

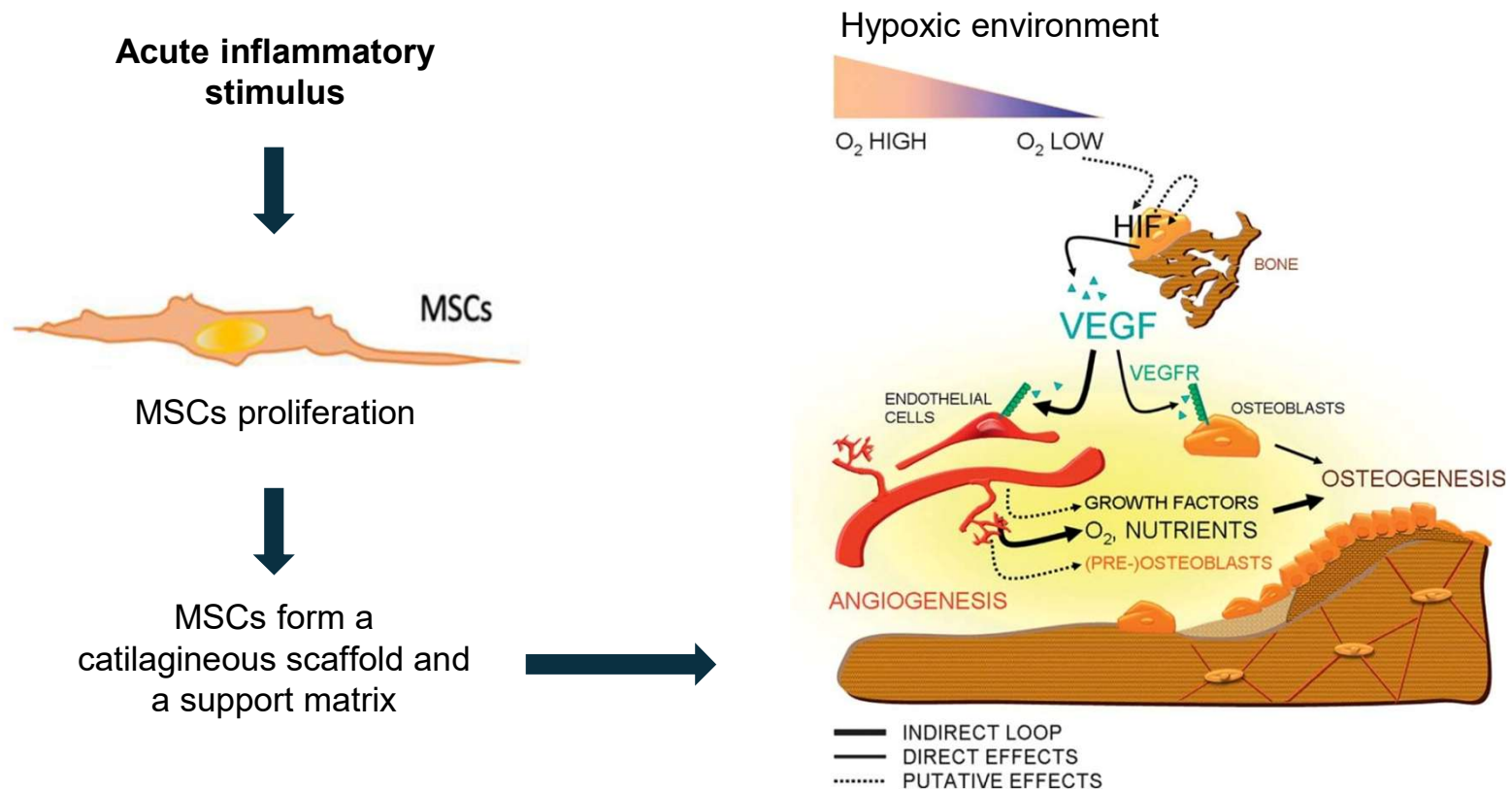
Silvia Brunelli

"Macrophage–FAP interactions in early heterotopic ossification in FOP: the emerging role of SPP1"

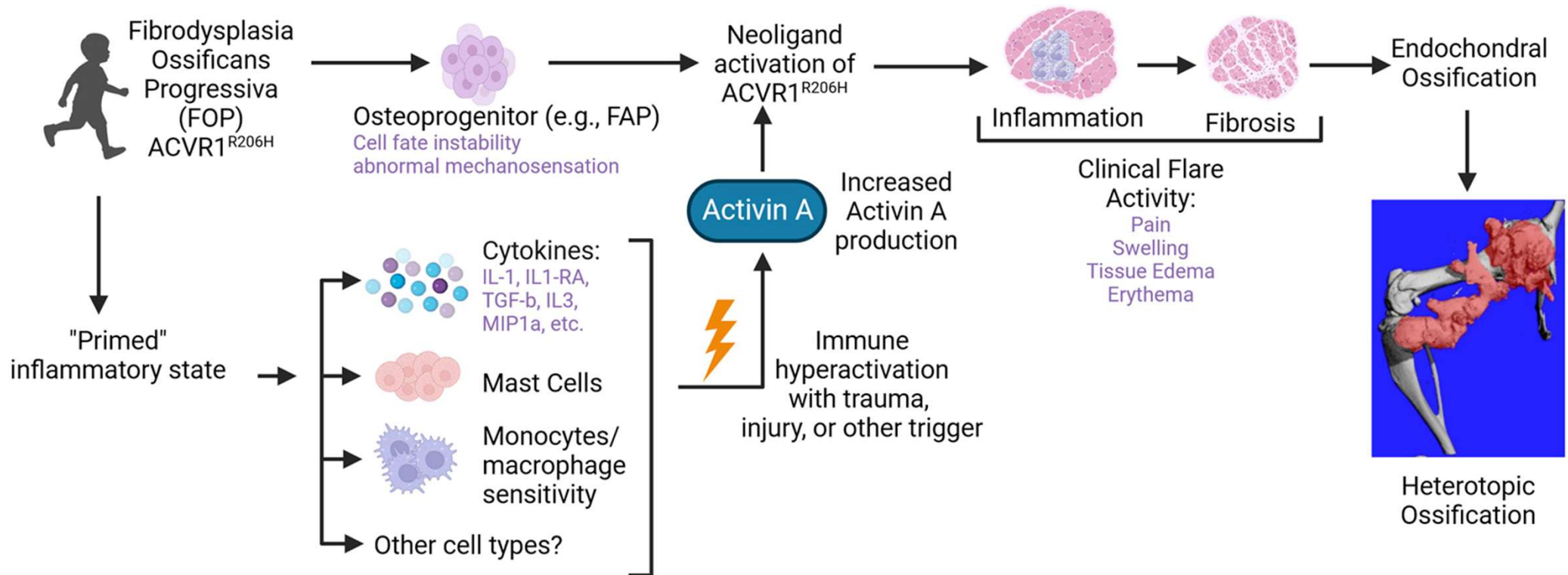
FOP Italia meeting
Livorno April 18th, 2026

Heterotopic Ossification (HO):

Formation of extraskeletal bone in skeletal muscle, joints and soft tissues, that follows an endochondral pathway

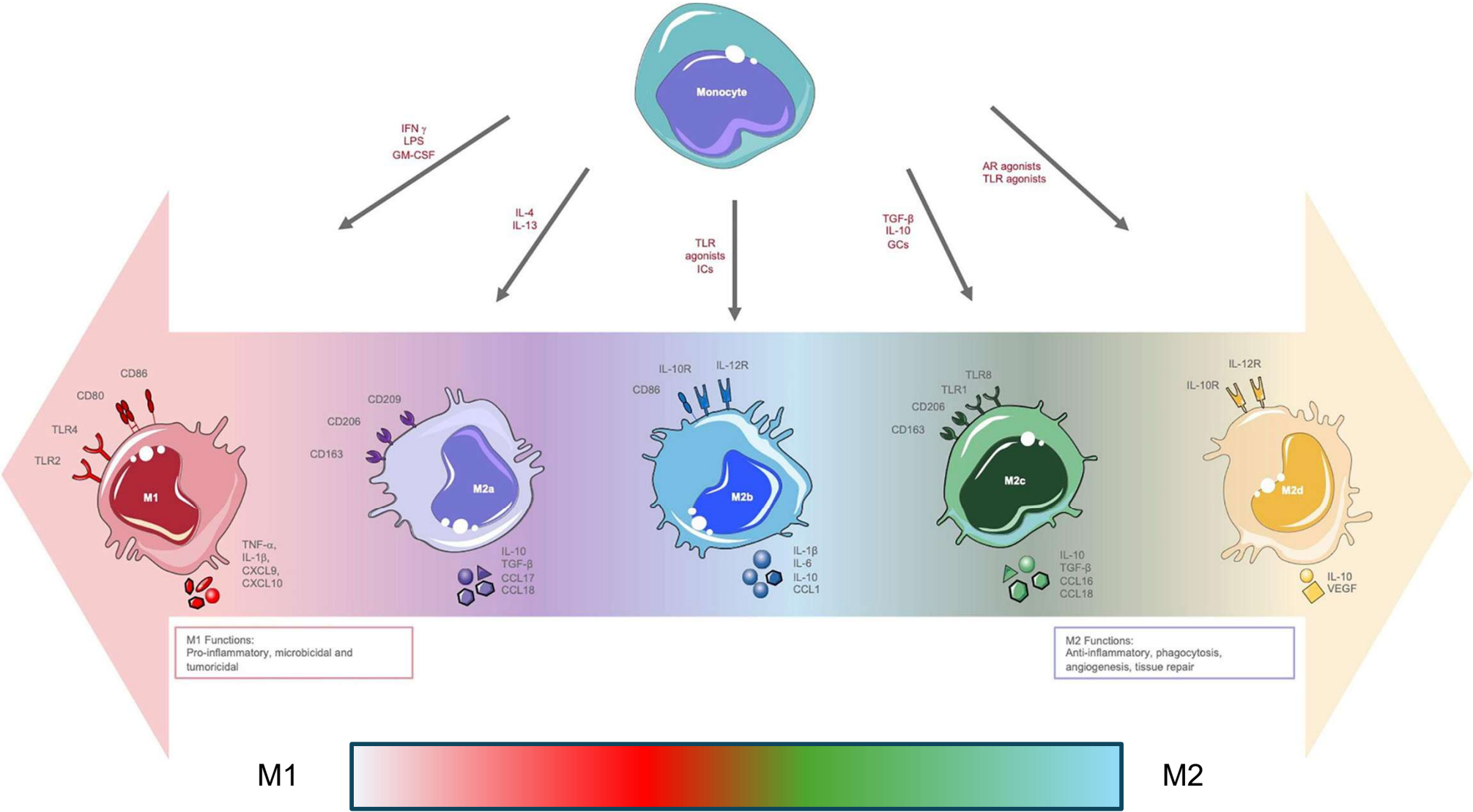


The immune system involvement in FOP

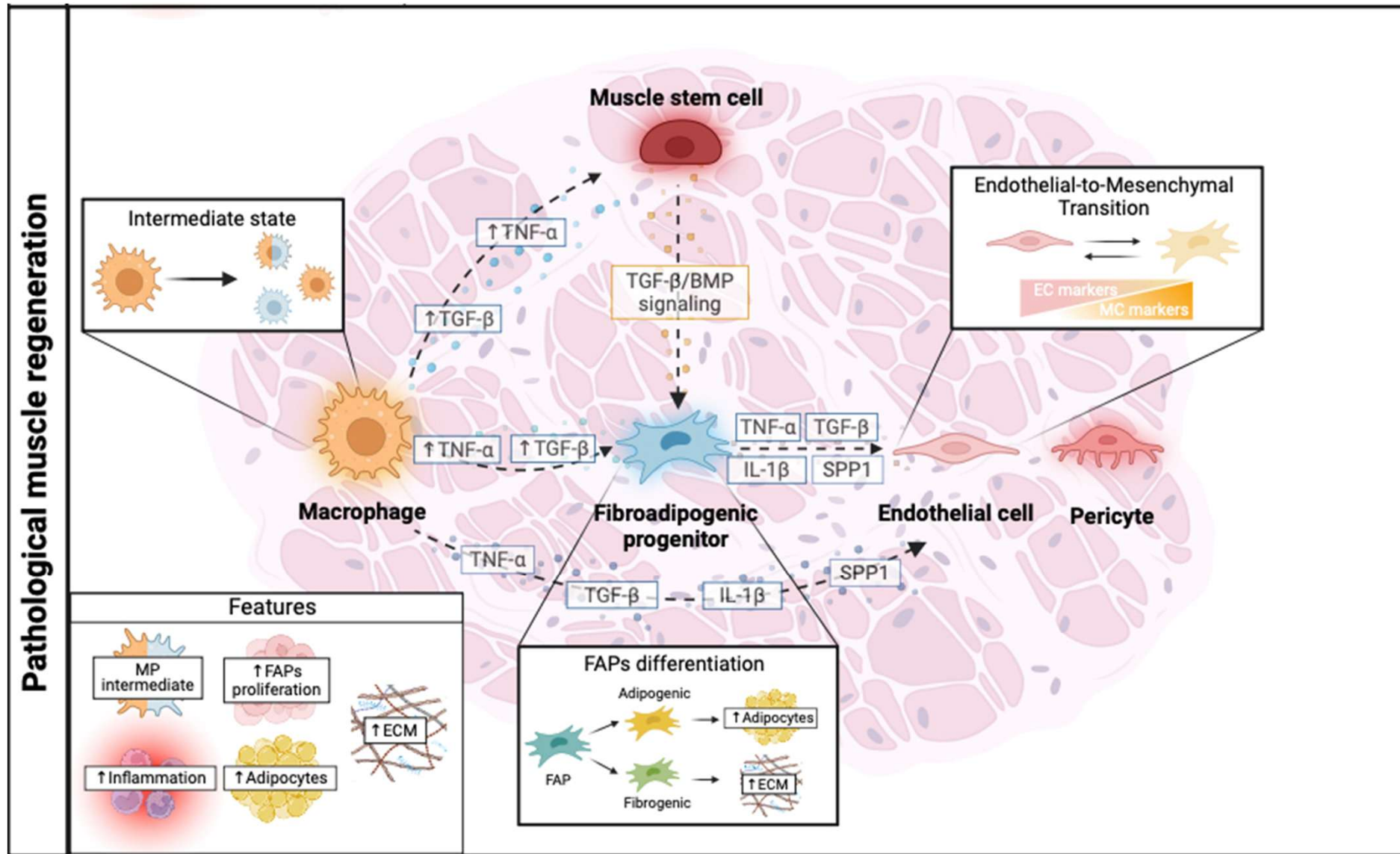


from Diolintzi et al, Biomolecules, 2024

Macrophages plasticity: One, No One and One Hundred Thousand



Macrophages in tissue regeneration

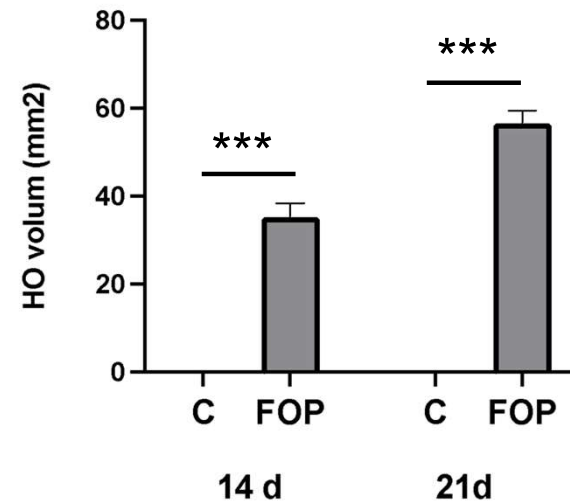
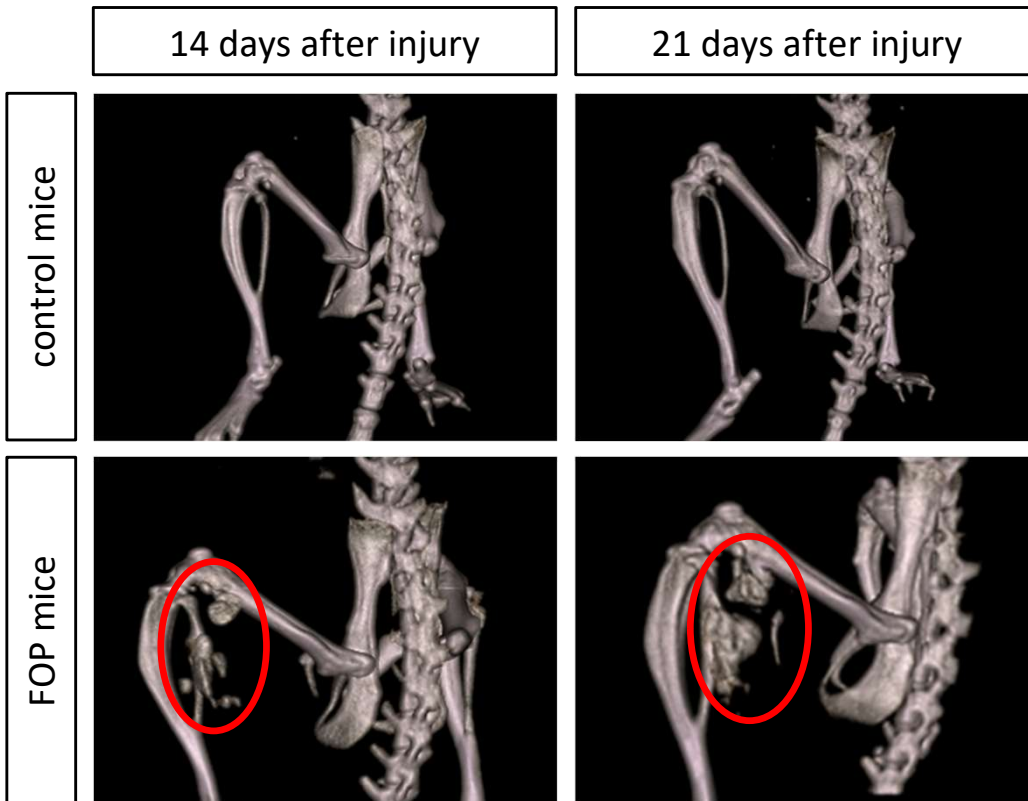
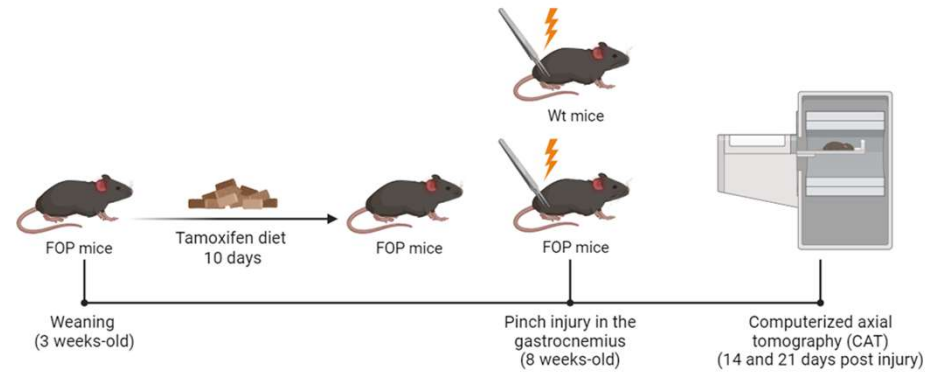
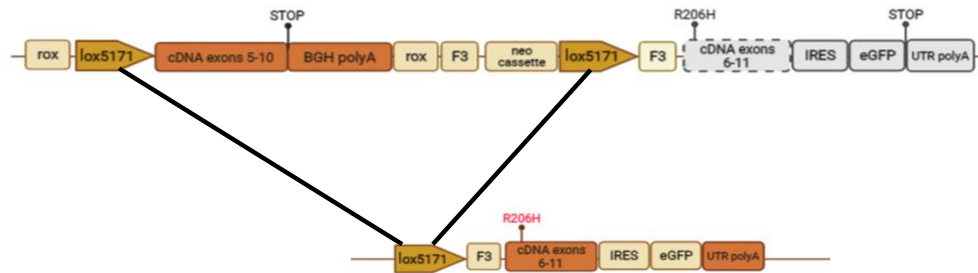


Rodriguez et al, 2024

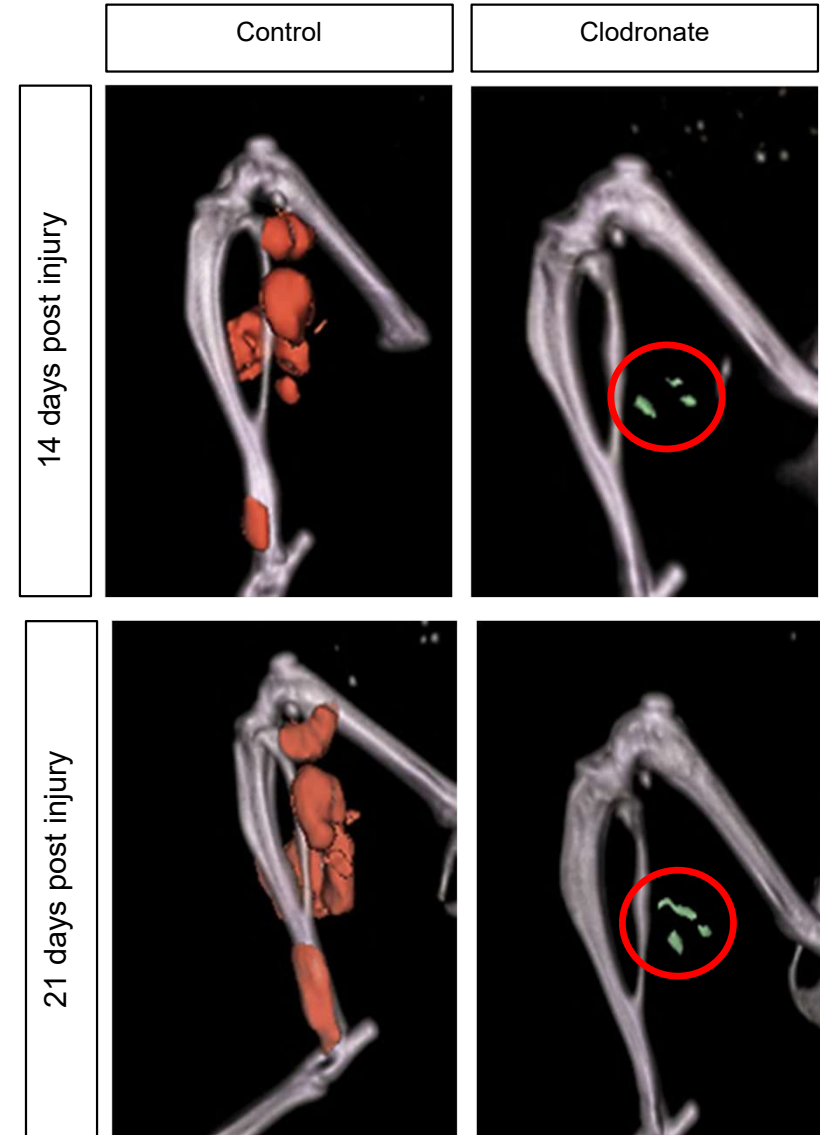
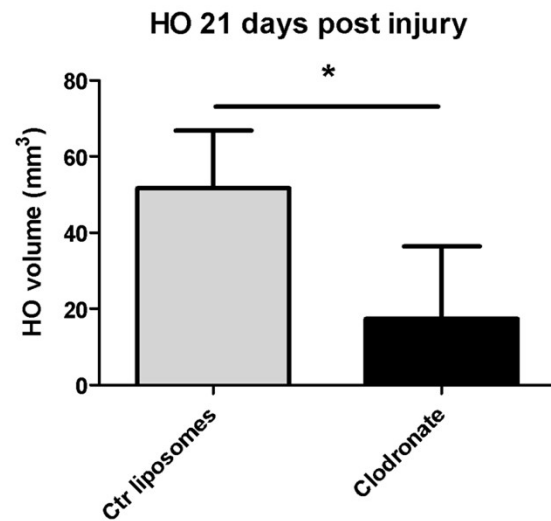
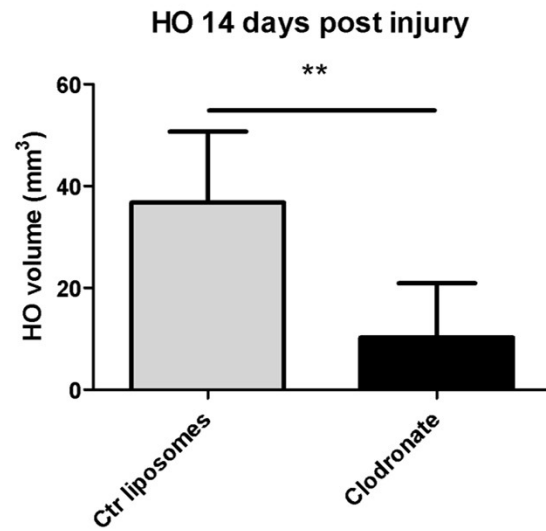
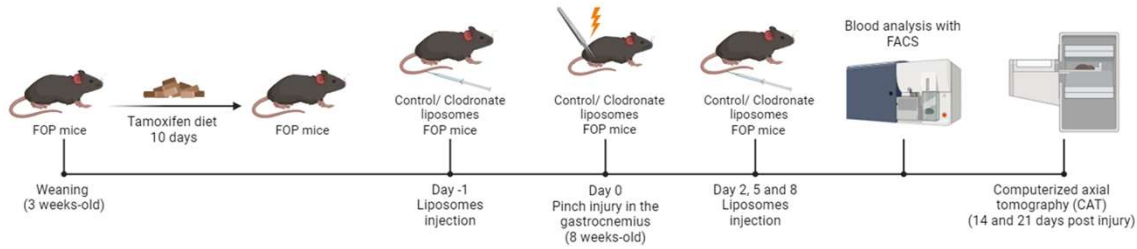
Chronic muscle injury

The FOP mouse model, Dan Perrien & IFOPA

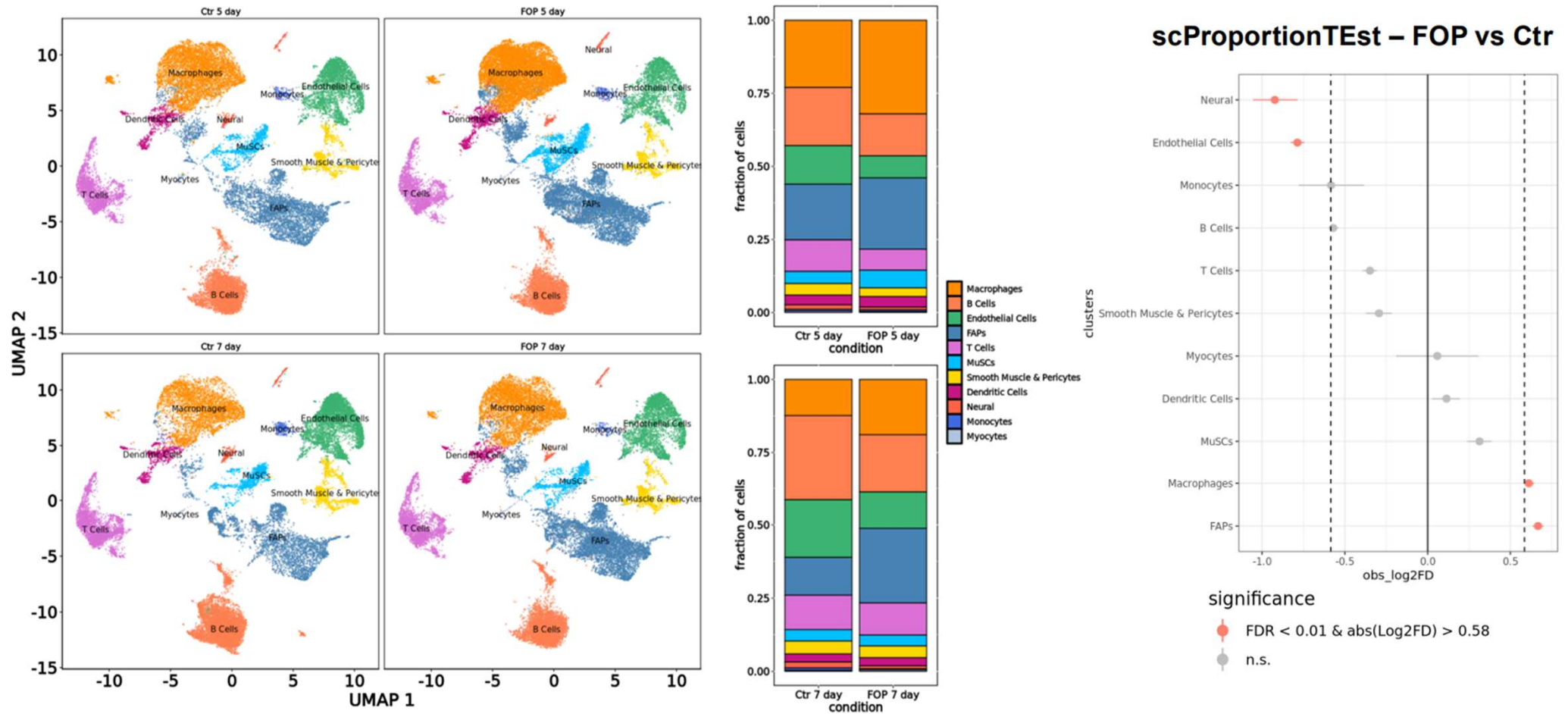
ALK2R206H^{fllox/-}: Gt(ROSA26)SorCre^{ERT2}



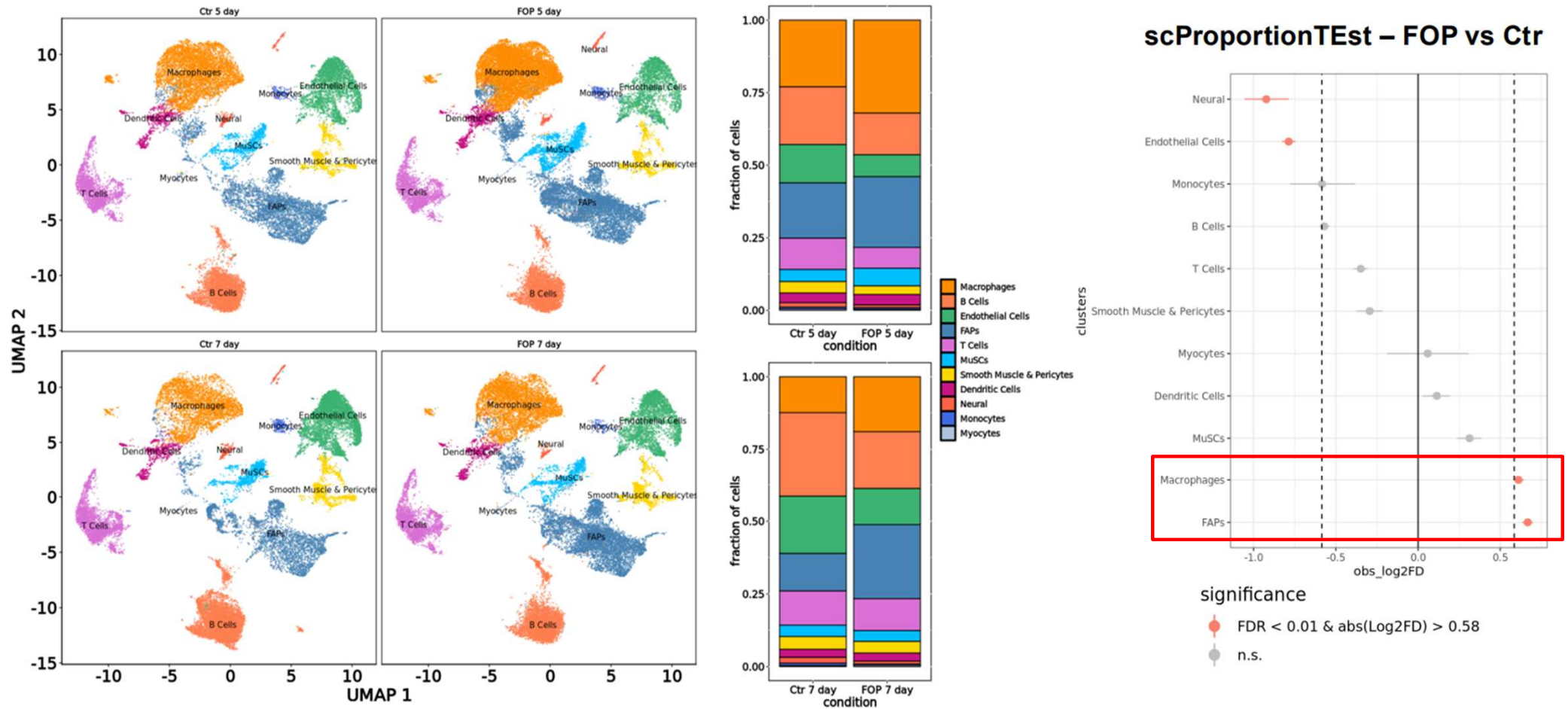
Clodronate decreases HO in ALK2^{R206H} mice



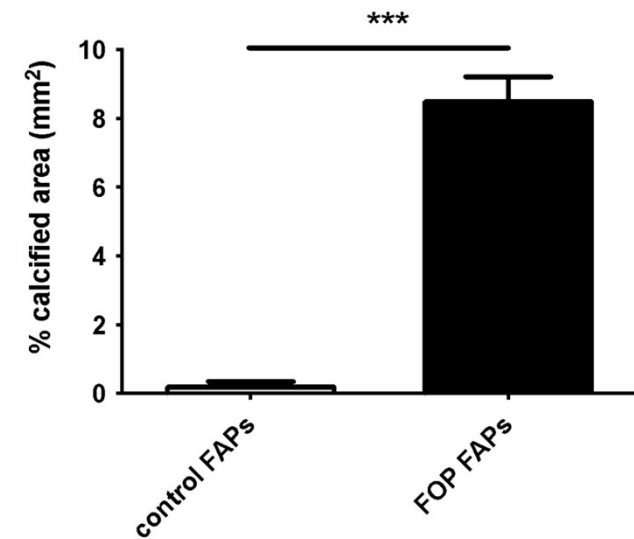
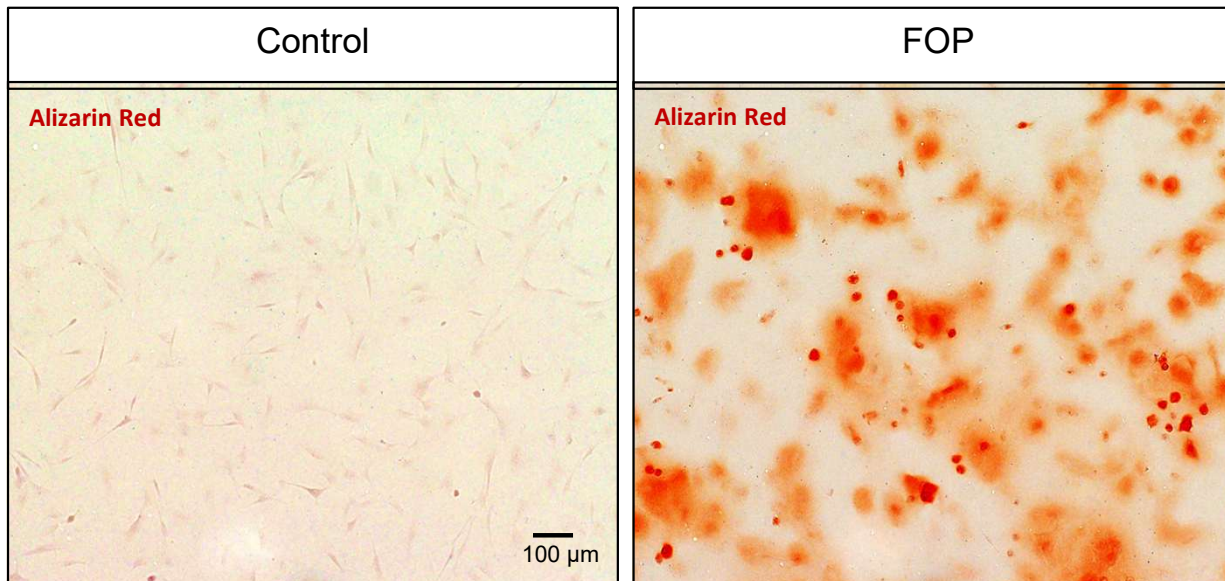
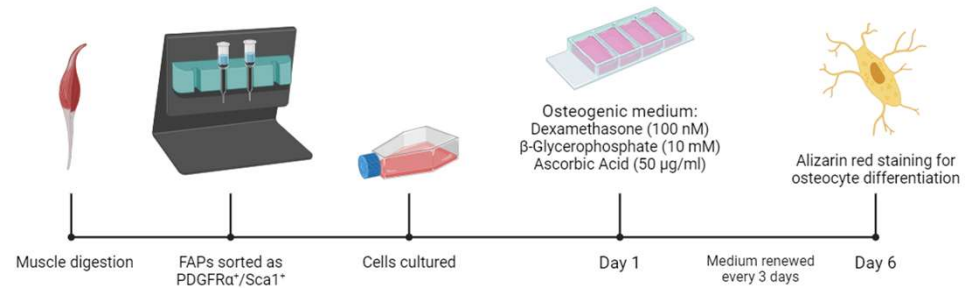
Single-cell RNA sequencing of early osteogenic lesions of the FOP mice shows altered cell composition



Single-cell RNA sequencing of early osteogenic lesions of the FOP mice shows altered cell composition

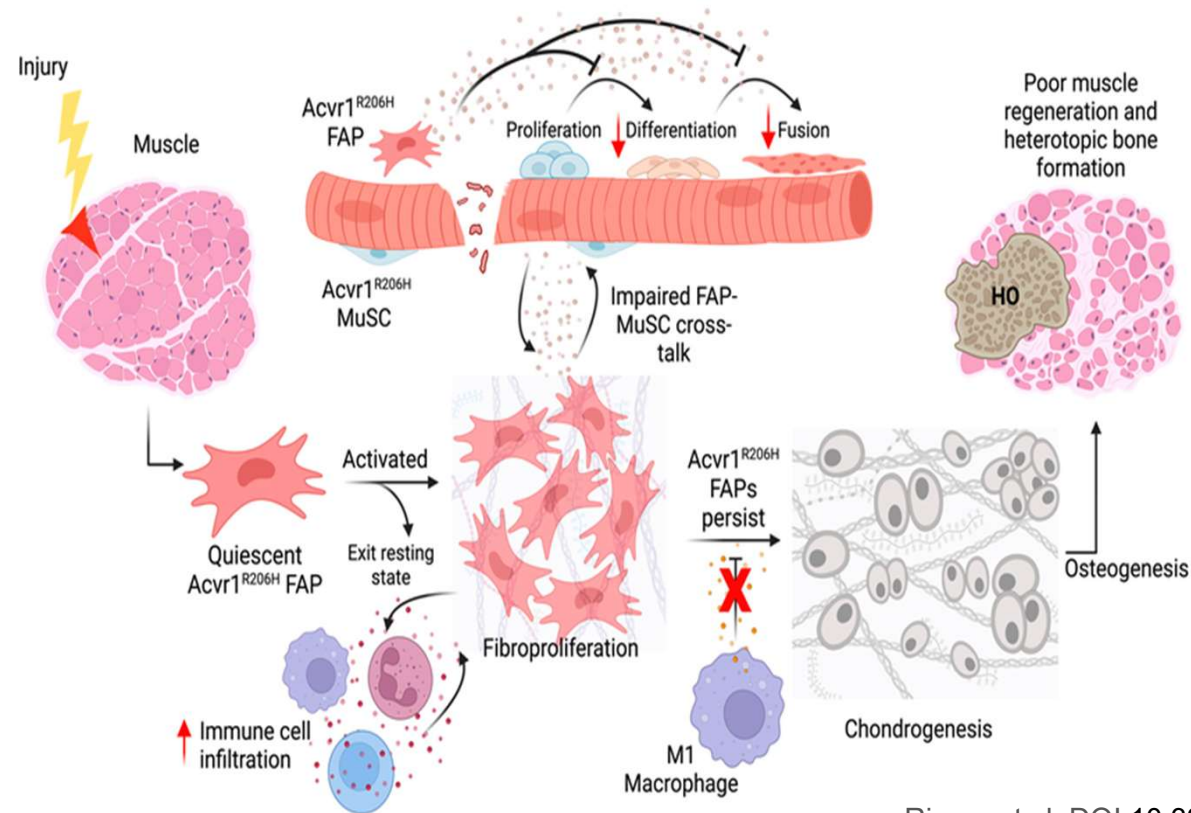


ALK2^{R206H} FAPs show faster osteogenic differentiation in vitro



Fibro-adipogenic progenitors (FAPs):

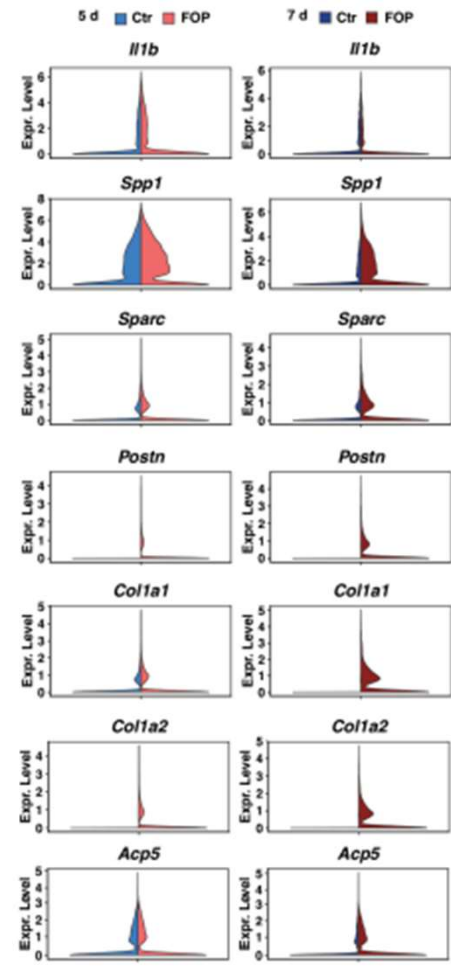
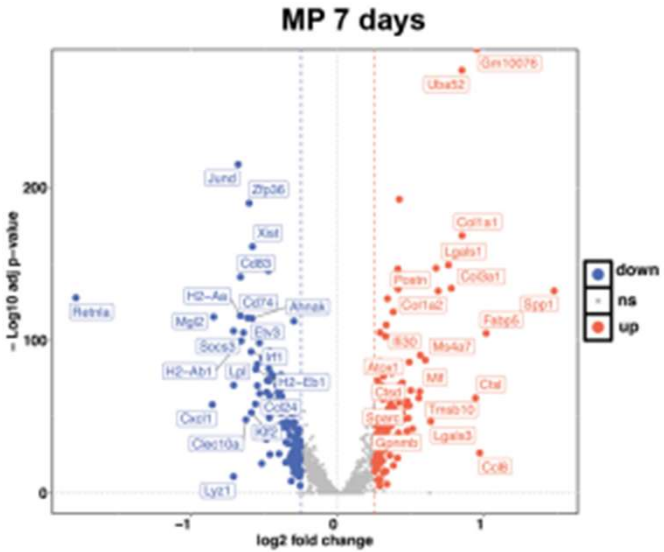
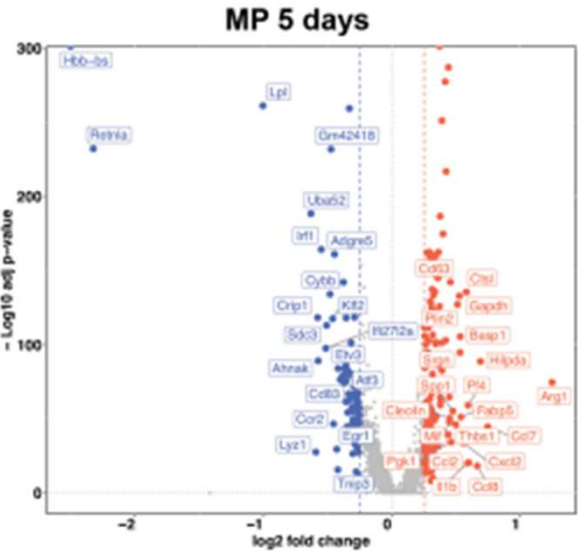
- Muscle-resident progenitors → adipogenic / myofibroblast fate
- Support repair: debris clearance + ECM deposition
- Key drivers of HO in FOP



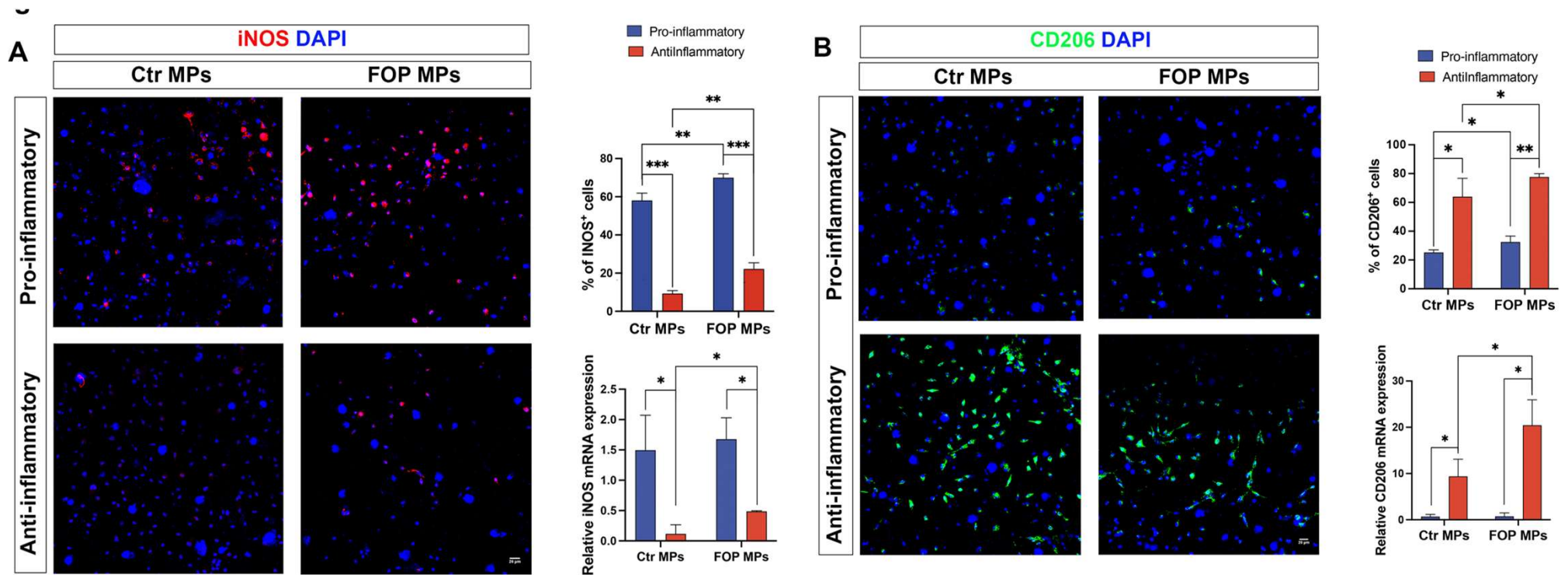
Rivera et al. DOI:[10.3390/biomedicines12040779](https://doi.org/10.3390/biomedicines12040779)

Which signals control their fate in FOP?

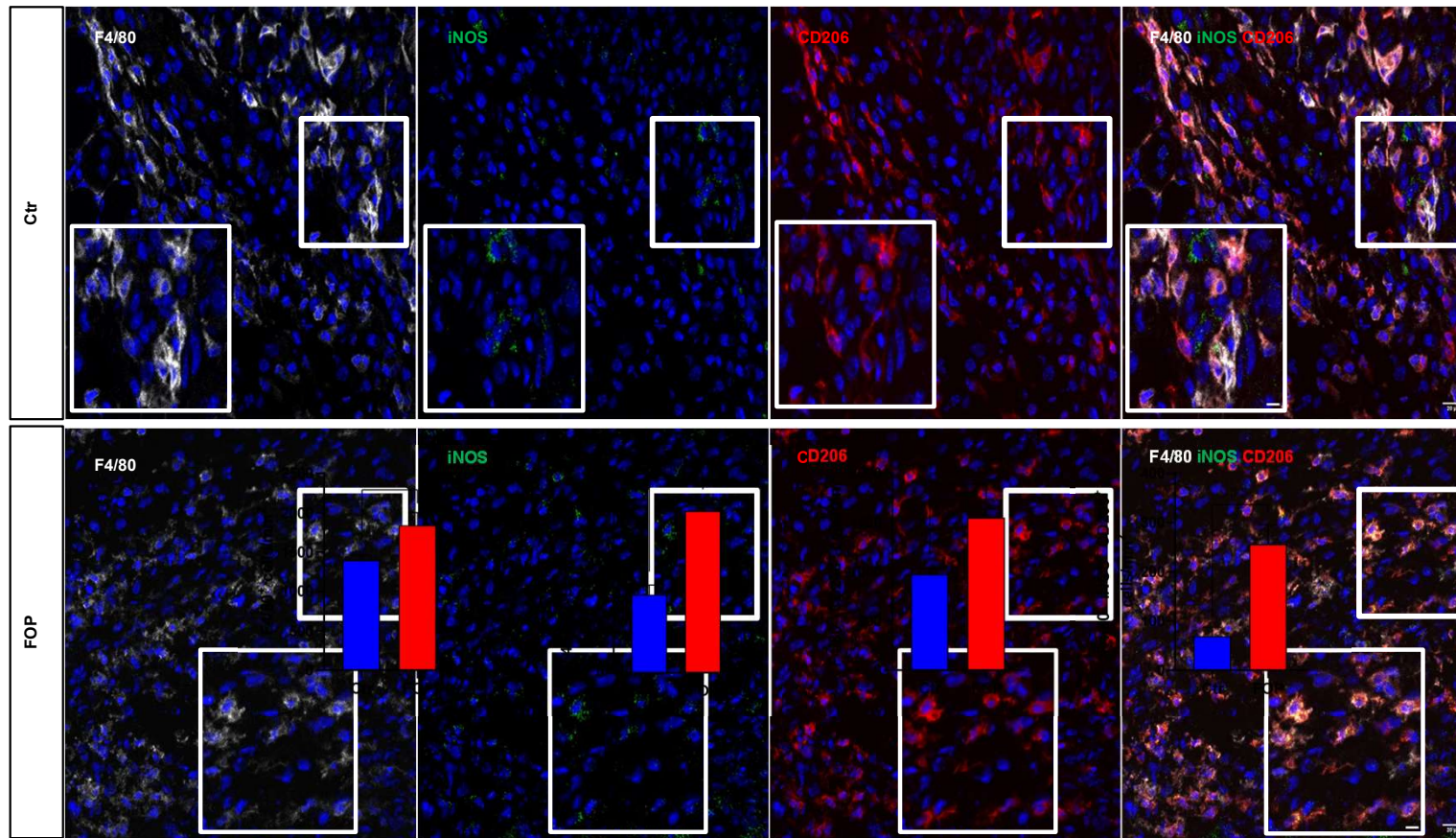
MPs displayed deregulated genes related to extracellular matrix remodelling and cellular metabolism



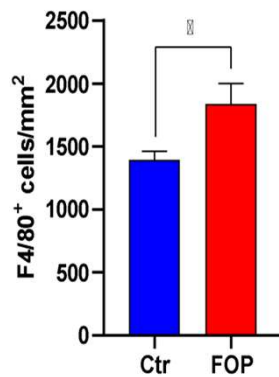
ALK2^{R206H} BMDMs display altered polarization in vitro



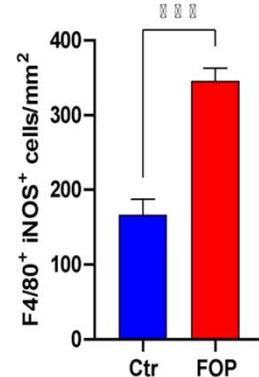
ALK2^{R206H} BMDMs display altered polarization *in vivo*



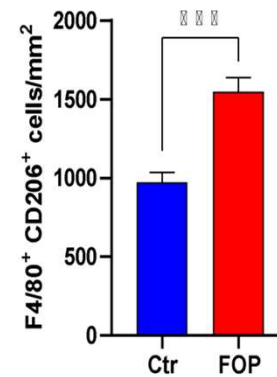
B



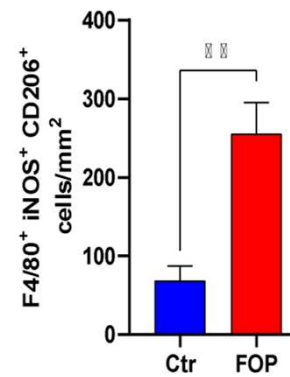
C



D

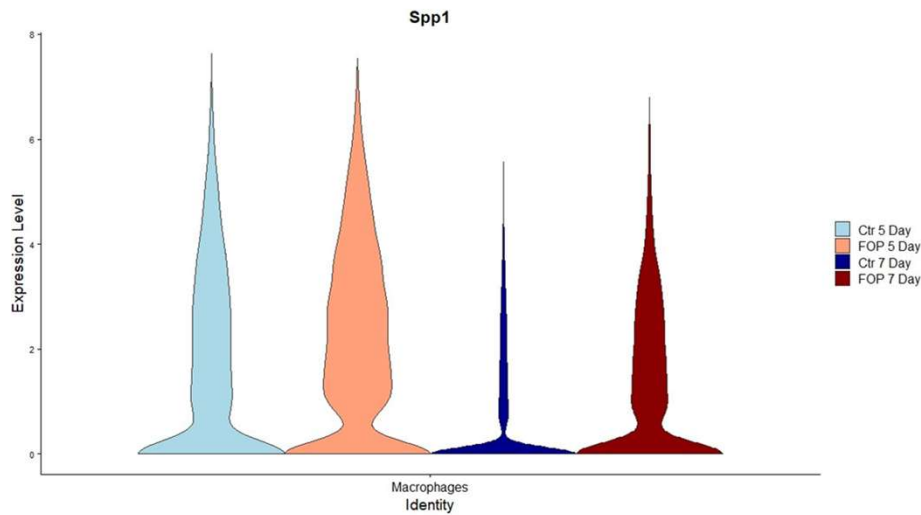


E

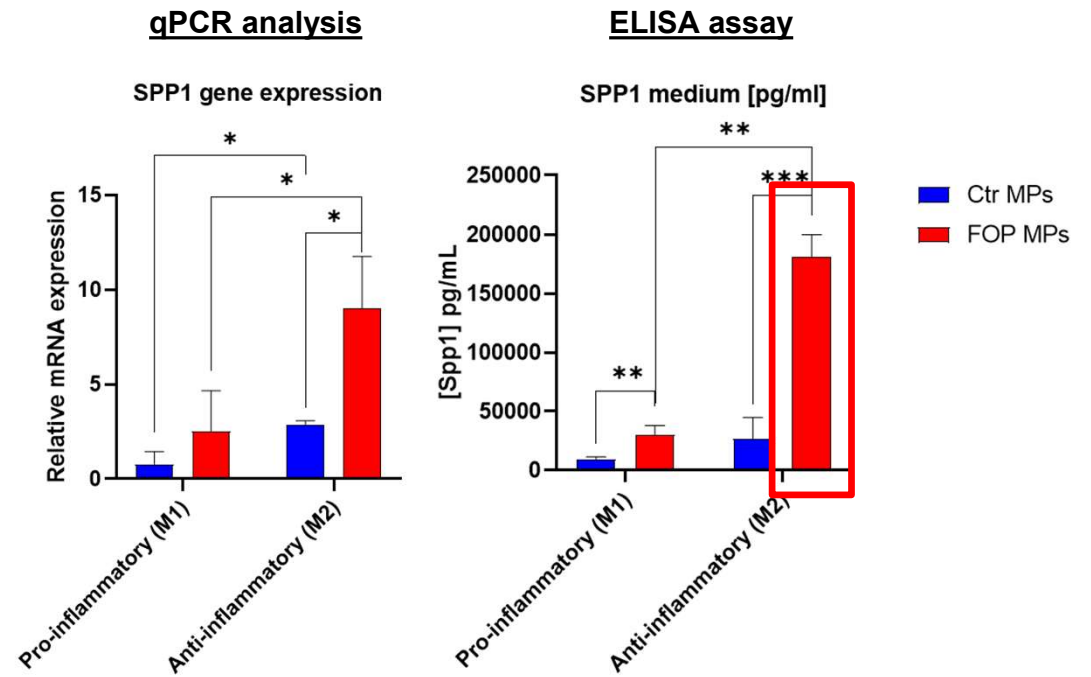


SPP1 is largely produced by anti-inflammatory MPs

Violin Plot of macrophage cluster for SPP1 gene

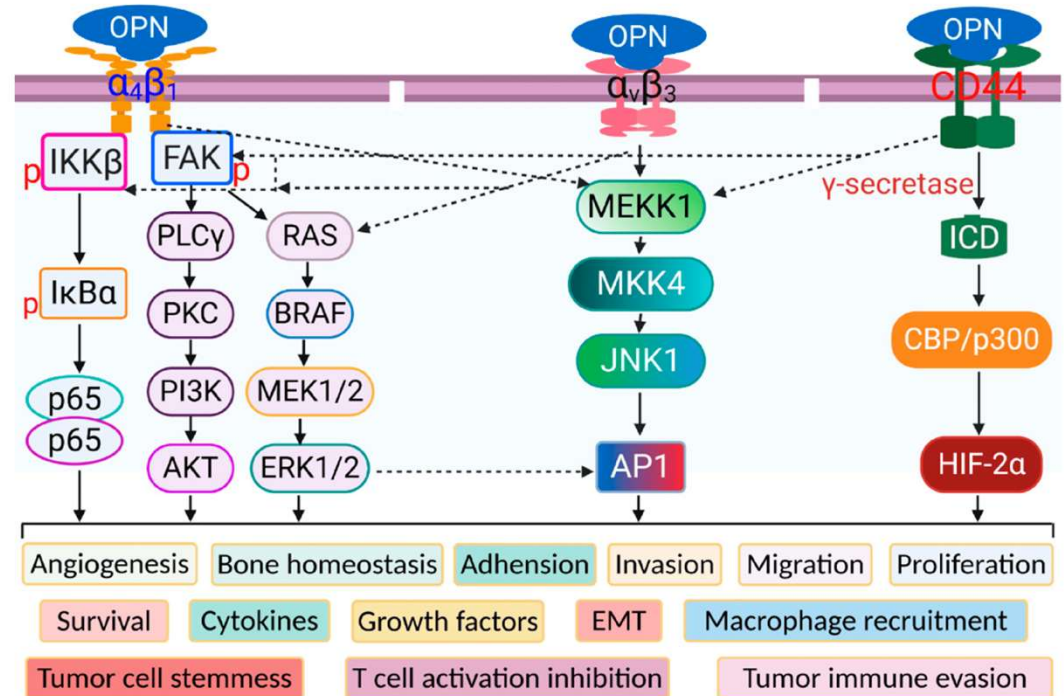
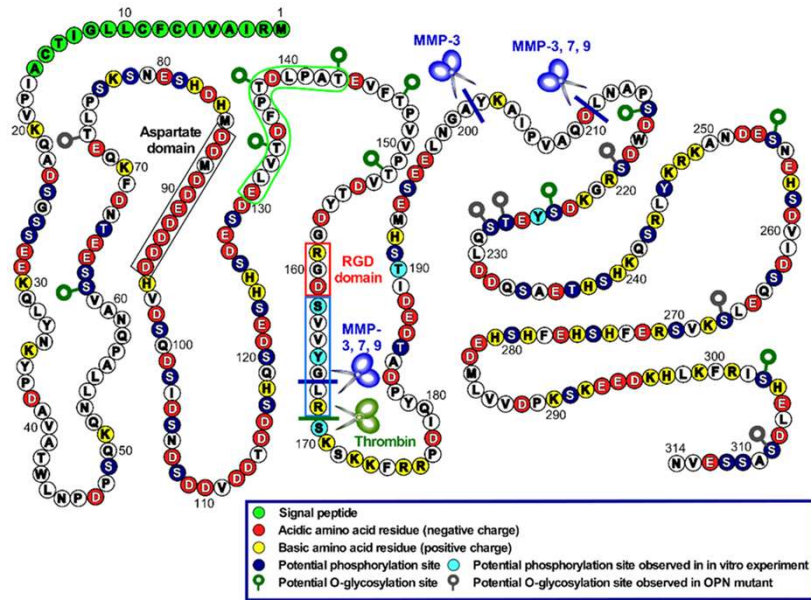


In vitro differentiation of MPs

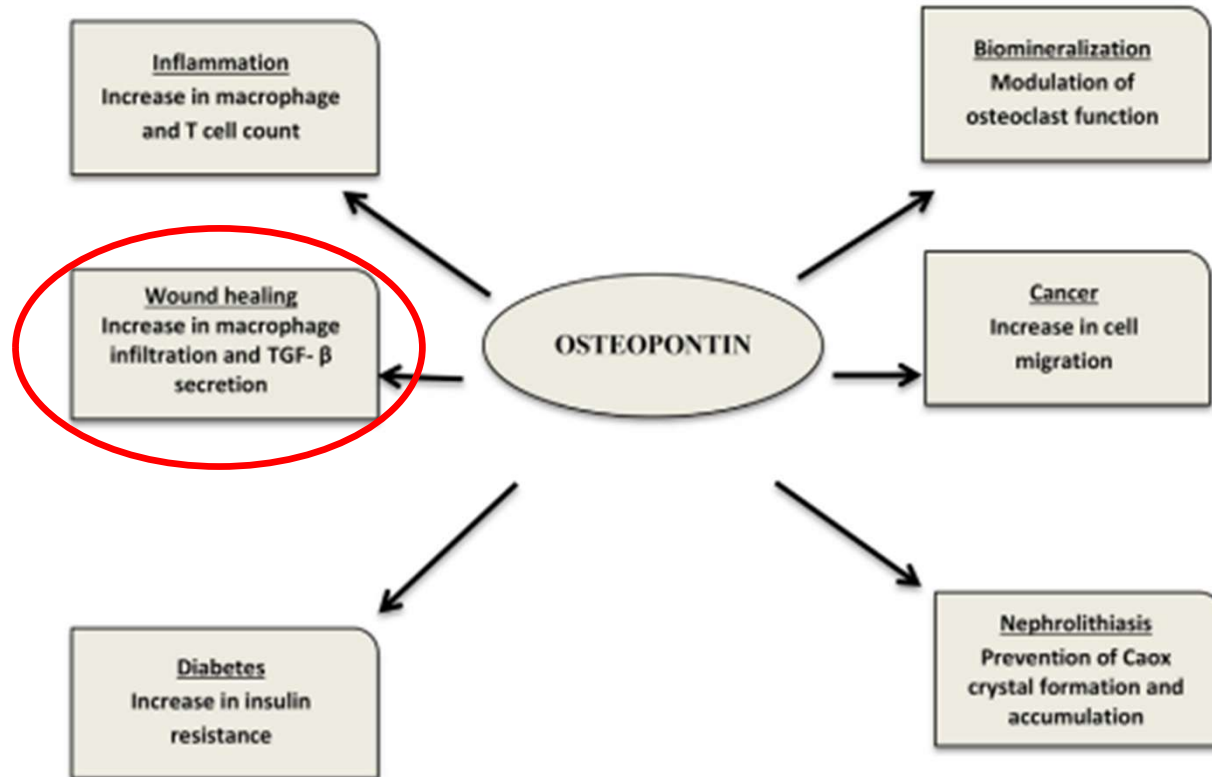


Osteopontin (Spp1)

- Osteopontin, or secreted phosphoprotein 1 (Spp1) is a highly phosphorylated glycoprotein consisting of 314 aminoacids, with a molecular weight ranging between 44 and 75 kDa



Biological function of Spp1



Icer & Gezmen-Karadag, *Clinical Biochemistry*. 2018

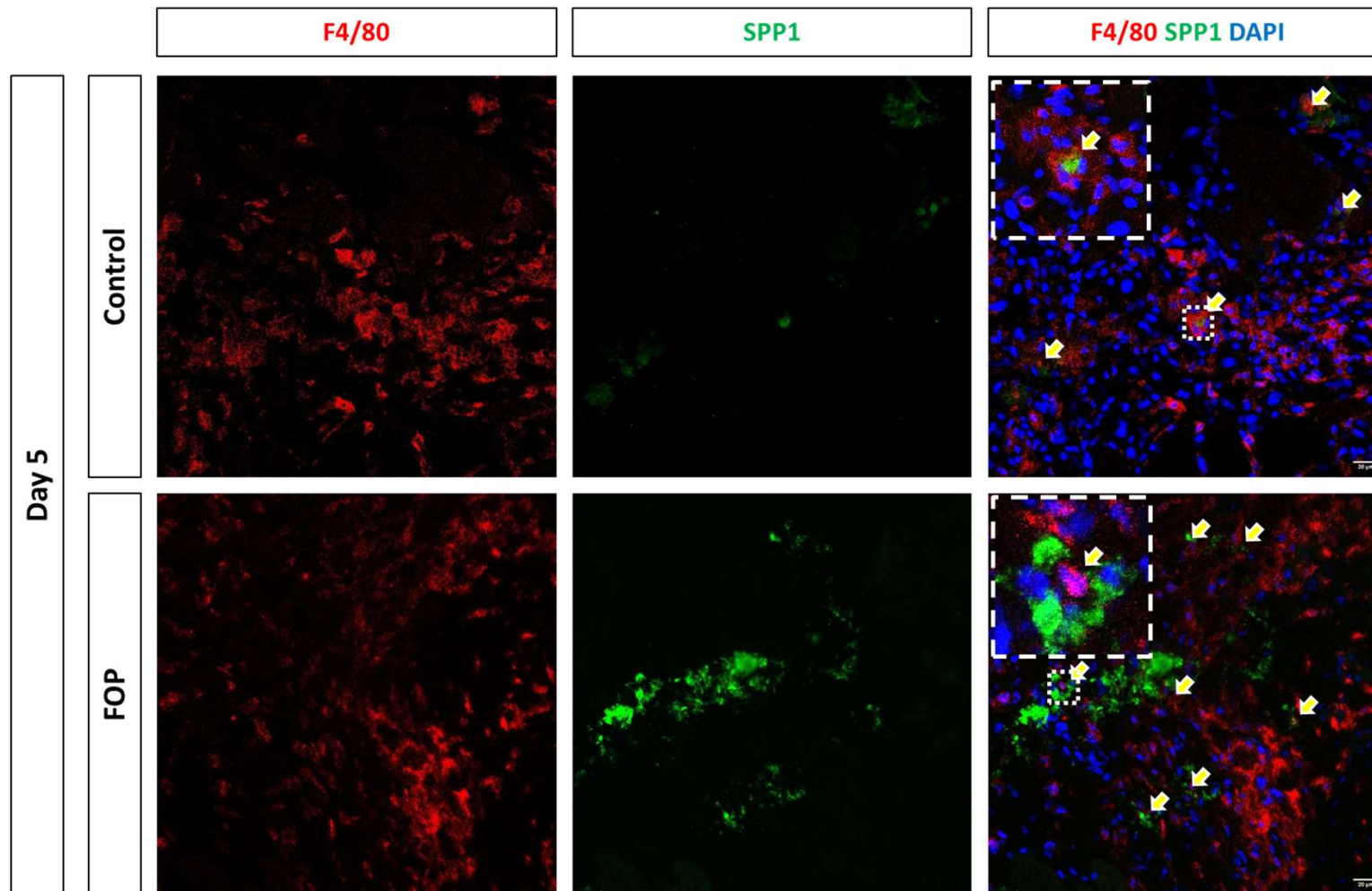
Spp1 (osteopontin) promotes TGF β processing in fibroblasts of dystrophin-deficient muscles through matrix metalloproteinases

Irina Kramerova¹, Chino Kumagai-Cresse^{1,2}, Natalia Ermolova¹, Ekaterina Mokhonova¹, Masha Marinov¹, Joana Capote^{1,3}, Diana Becerra¹, Mattia Quattrocelli², Rachelle H Crosbie^{1,4,5}, Ellen Welch⁶, Elizabeth M McNally^{2,5}, Melissa J Spencer^{1,5}

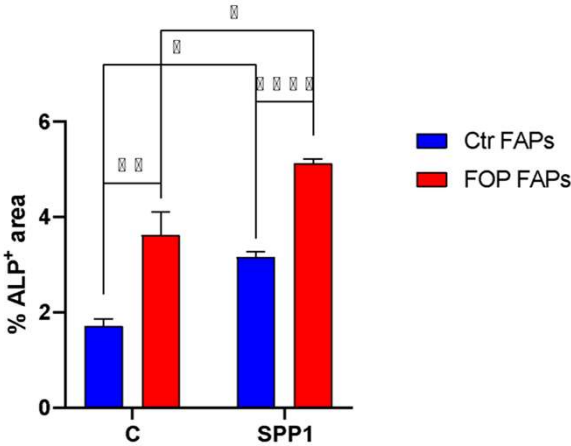
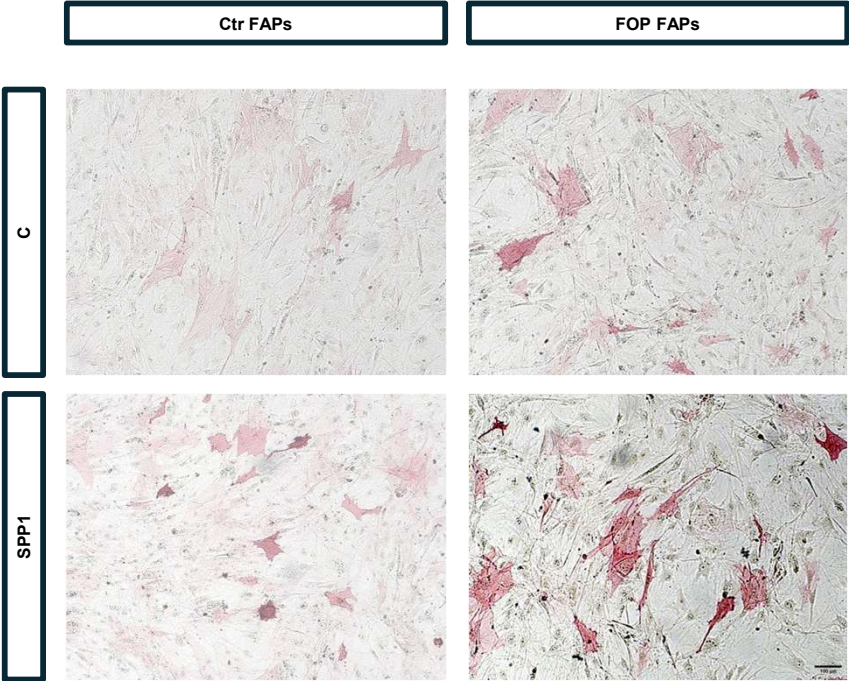
> *Nat Commun*. 2022 Apr 1;13(1):1742. doi: 10.1038/s41467-022-29366-6.

Single-cell and spatial analysis reveal interaction of FAP⁺ fibroblasts and SPP1⁺ macrophages in colorectal cancer

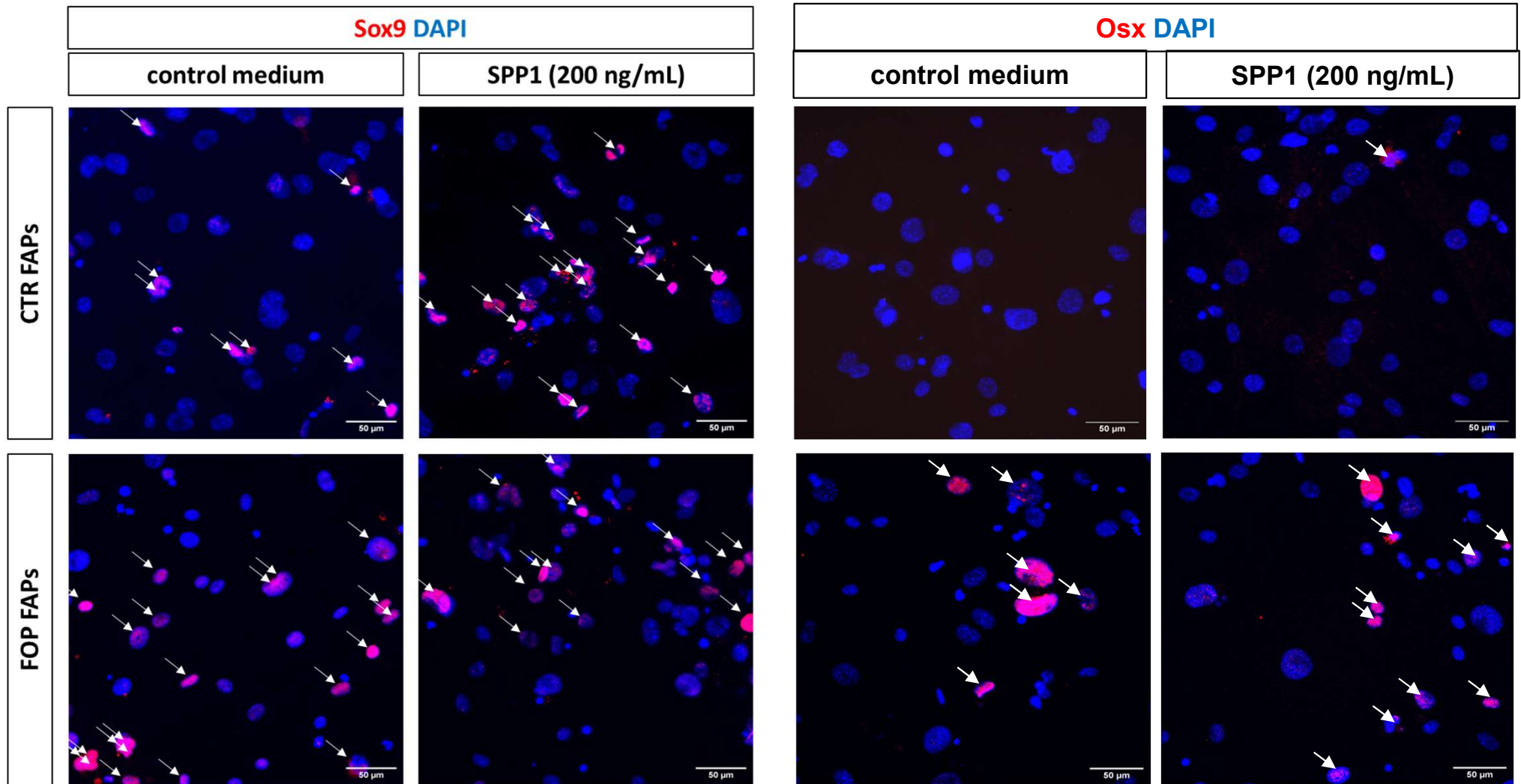
Spp1 expression in MPs of ALK2^{R206H} mice



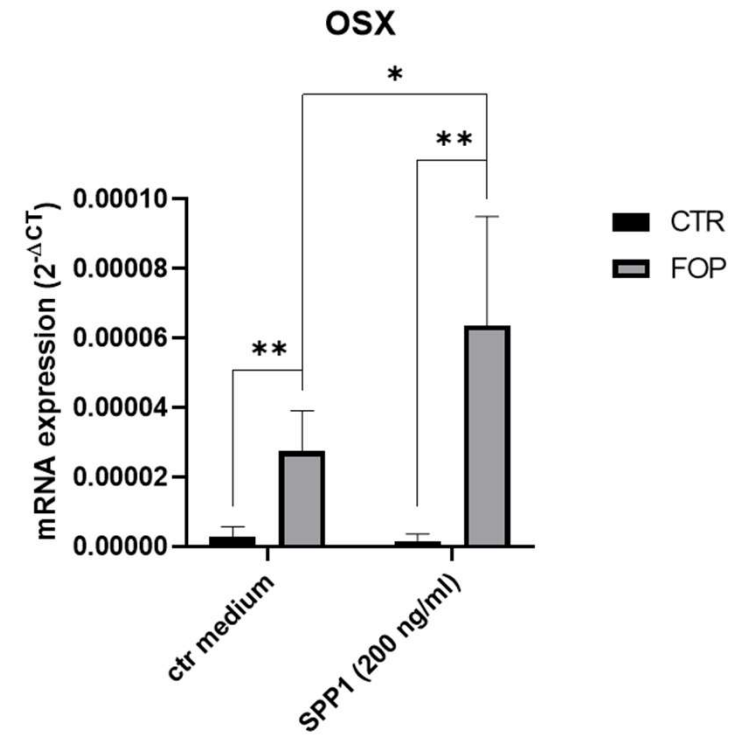
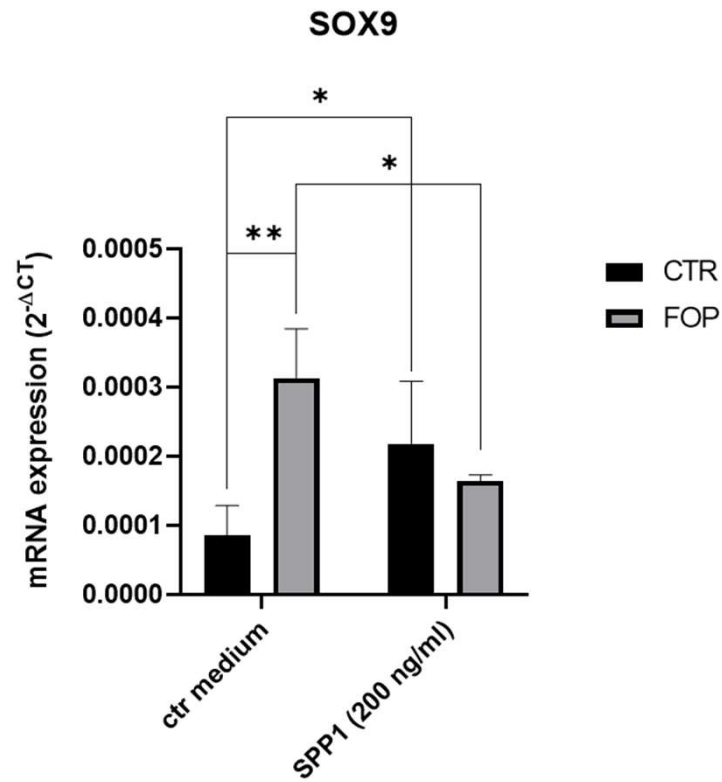
SPP1 enhances osteogenic differentiation in ALK2^{R206H} FAPs



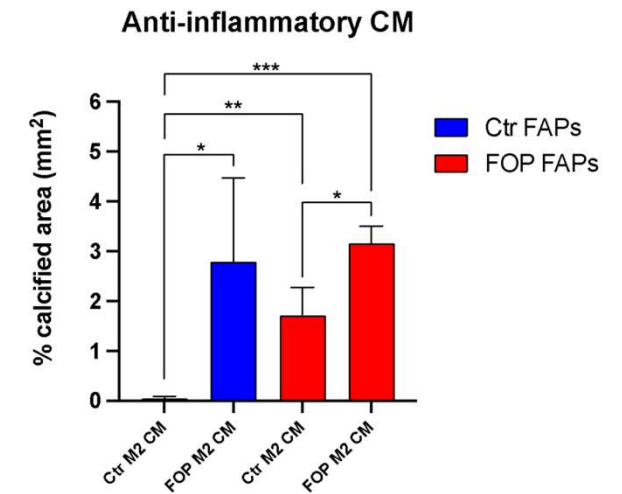
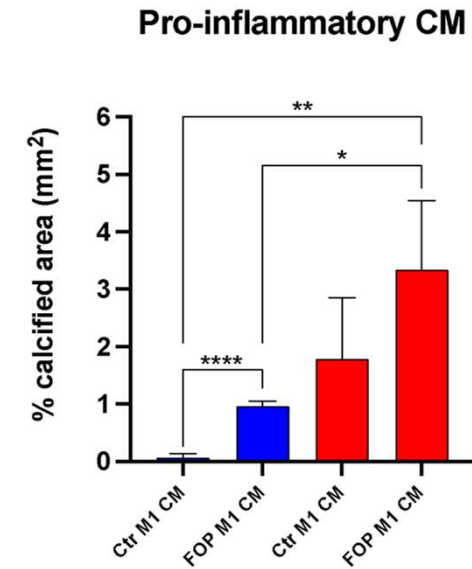
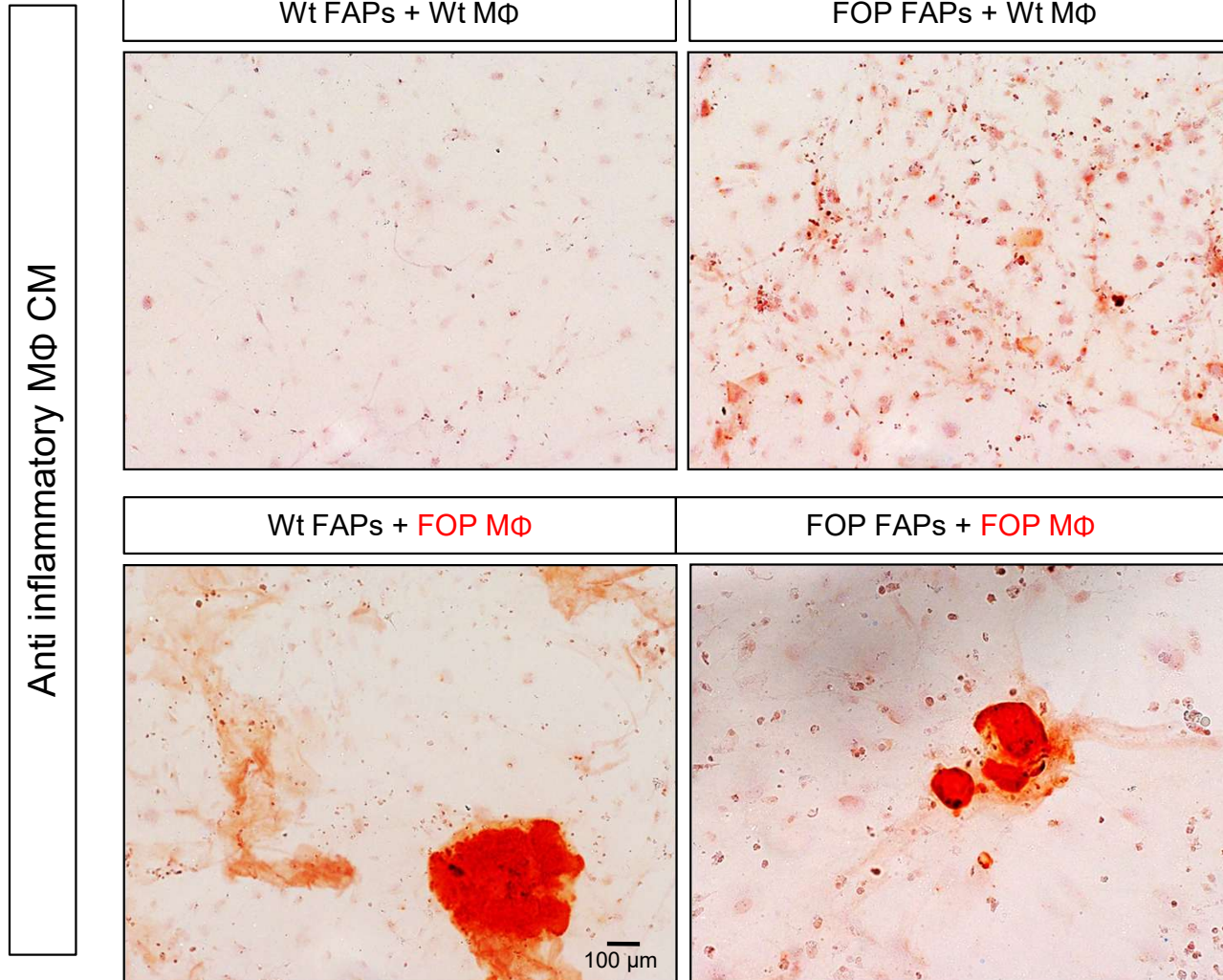
SPP1 enhances osteocyte differentiation in $ALK2^{R206H}$ FAPs



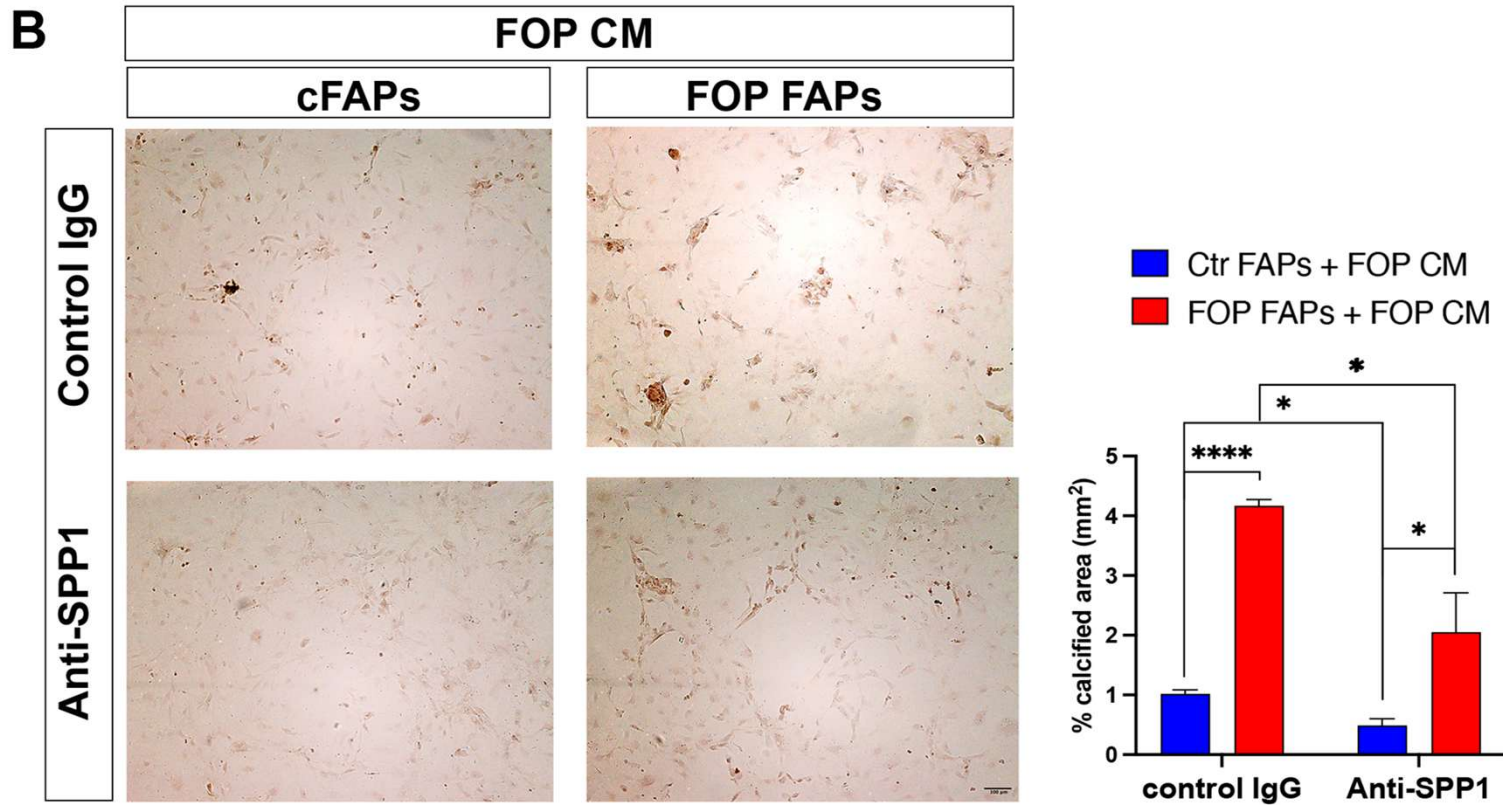
SPP1 enhances osteocyte differentiation in ALK2^{R206H} FAPs



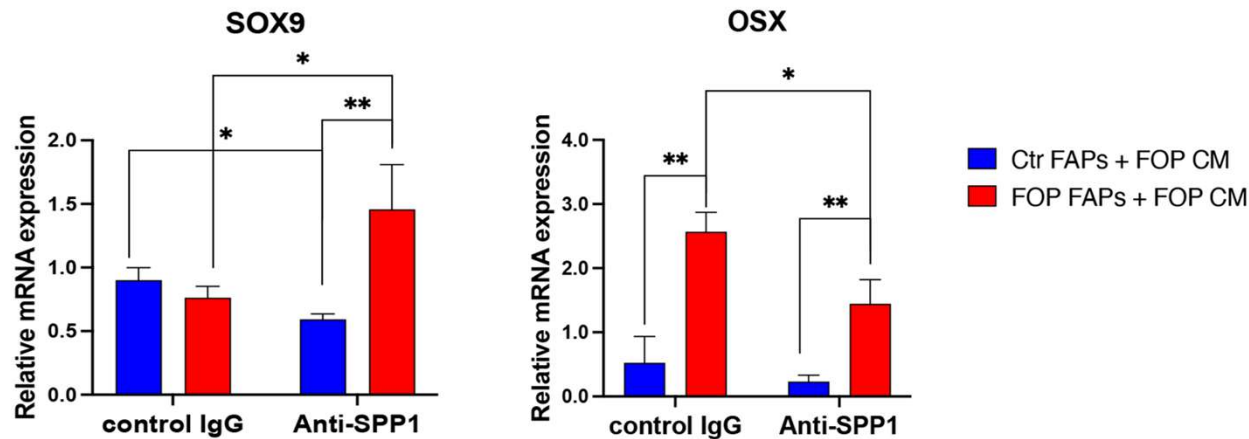
FOP pro and anti-inflammatory MΦ CM promotes FAPs osteogenesis in FOP and Control mice



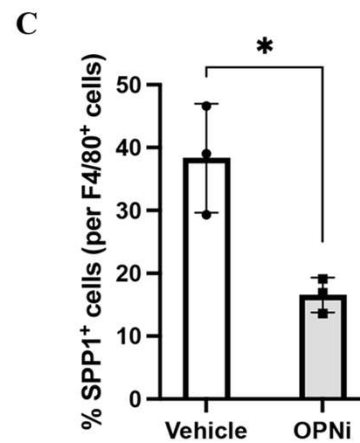
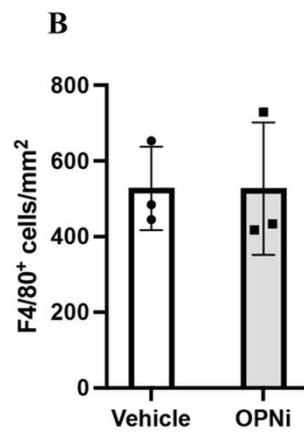
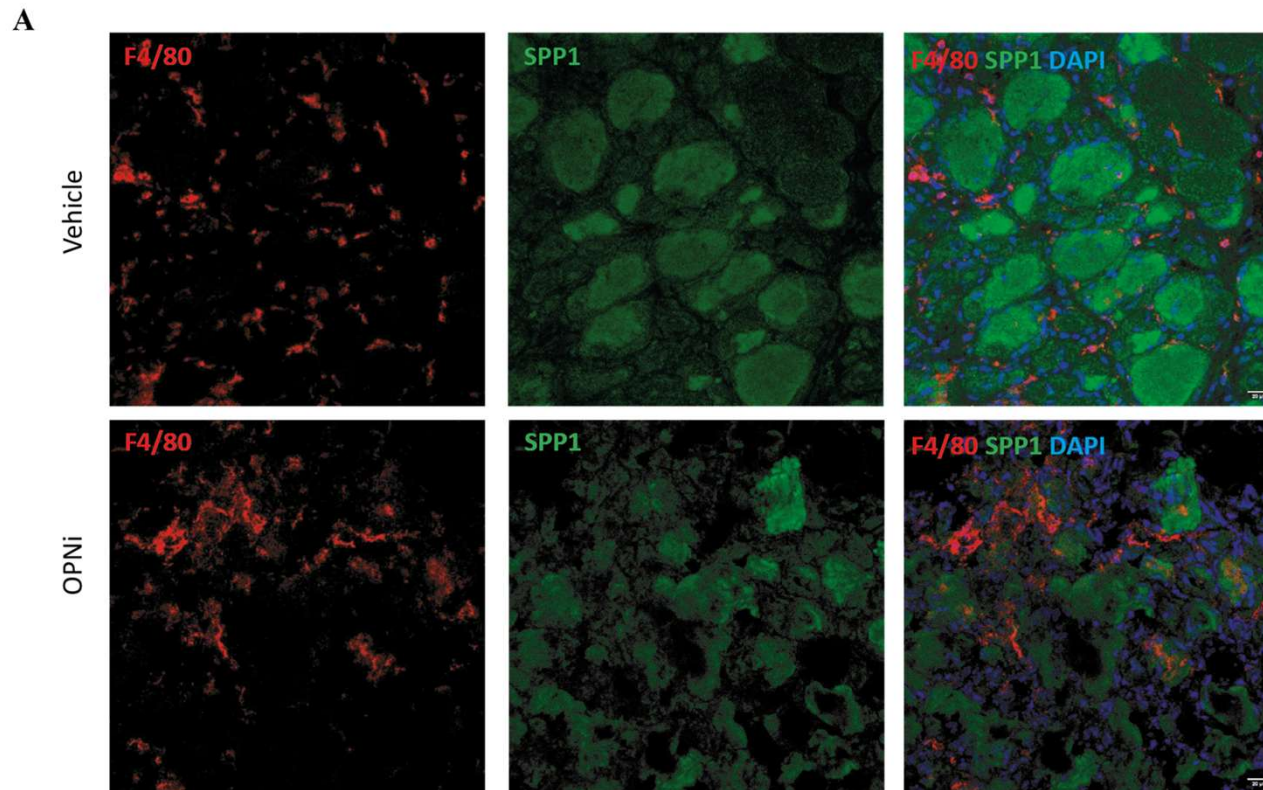
SPP1 inhibition decreases FOP FAP osteogenic differentiation



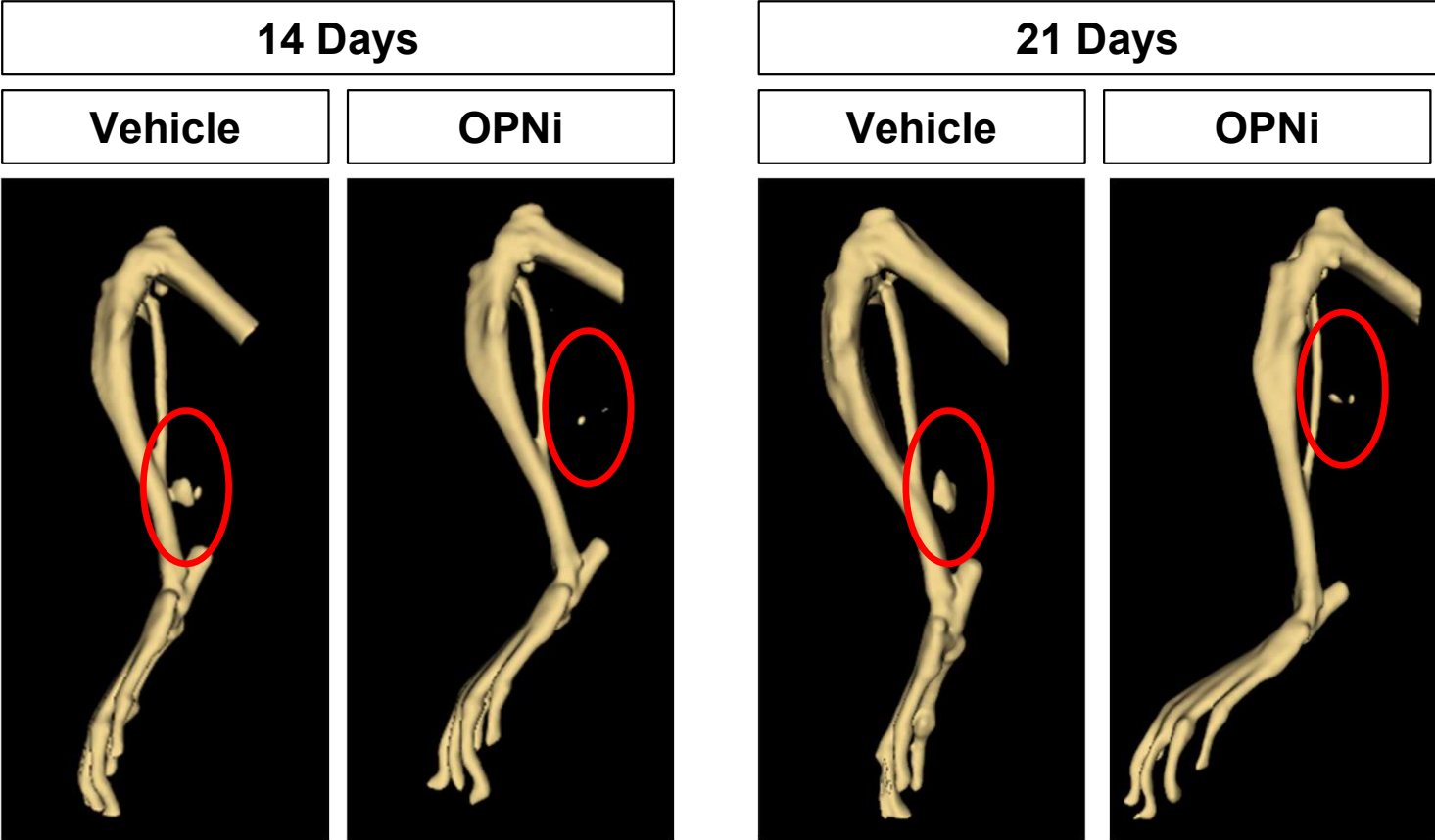
C



Pharmacological inhibition of SPP1 in vivo

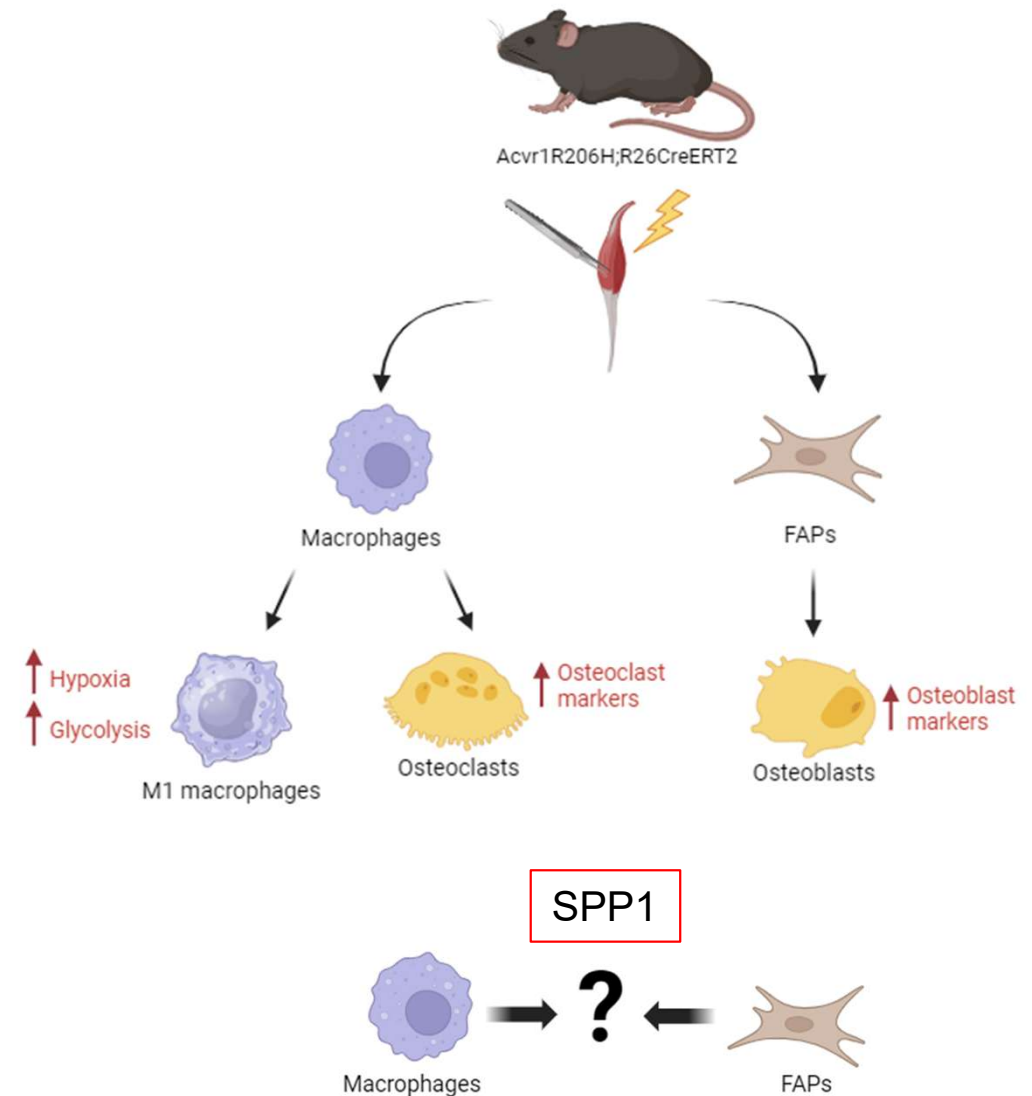


Preliminary data: pharmacological inhibition of SPP1 decrease HO in ALK2^{R206H}



Conclusions

- Macrophage depletion in $Acvr1^{R206H/lox/lox};Gt(ROSA26)SorCre$ ERT2 mice using clodronate impairs HO development
- Single-cell transcriptomics show an unbalanced macrophage metabolism and polarization in the FOP mouse model
- Single-cell transcriptomics and in vitro assays suggests that in the FOP mouse model macrophages and FAPs are committed to form a cellular niche that promotes and sustains bone formation
- Macrophage derived SPP1 appears to play a crucial role in enhancing HO in vitro and in vivo



Next steps

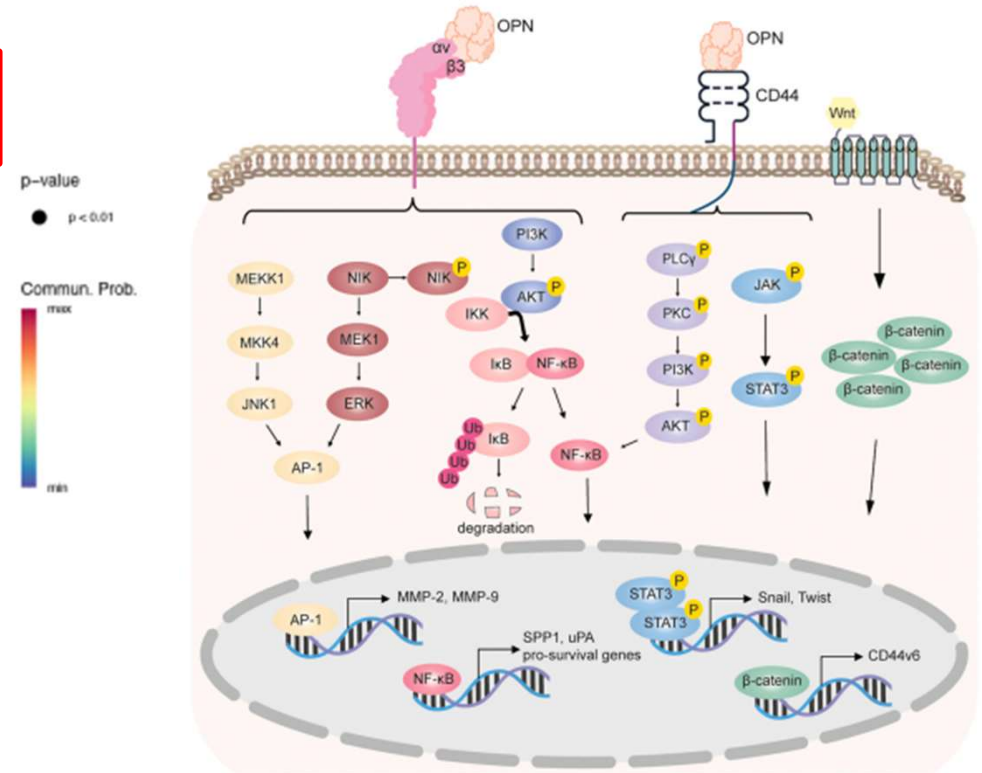
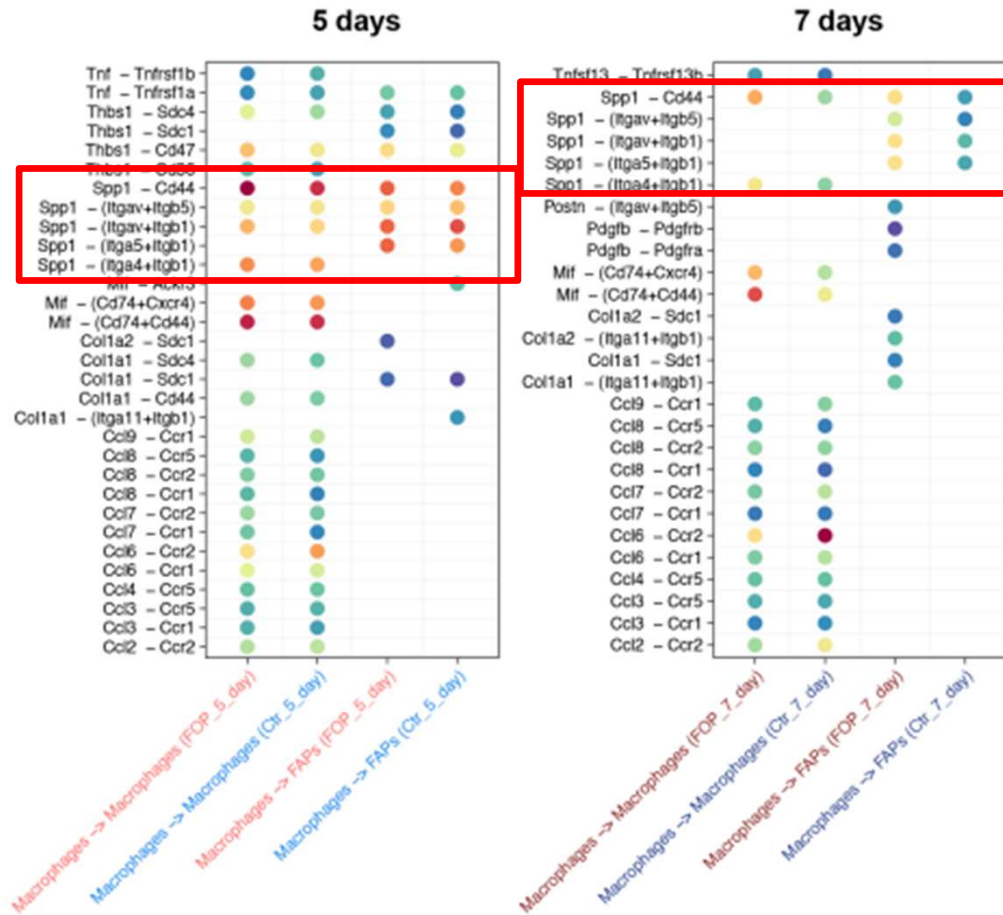
- Characterize SPP1 role on $ALK2^{R206H}$ MPs polarization and on their crosstalk with chondro-osteogenic precursors (FAPs) using genetic or pharmacological silencing

siRNA inhibition of SPP1 in a macrophage cell line (RAW 264.7)

Next steps

- Characterize SPP1 role on $ALK2^{R206H}$ MPs polarization and on their crosstalk with chondro-osteogenic precursors (FAPs)
- To explore SPP1 and related inflammatory pathways as potential therapeutic targets for FOP

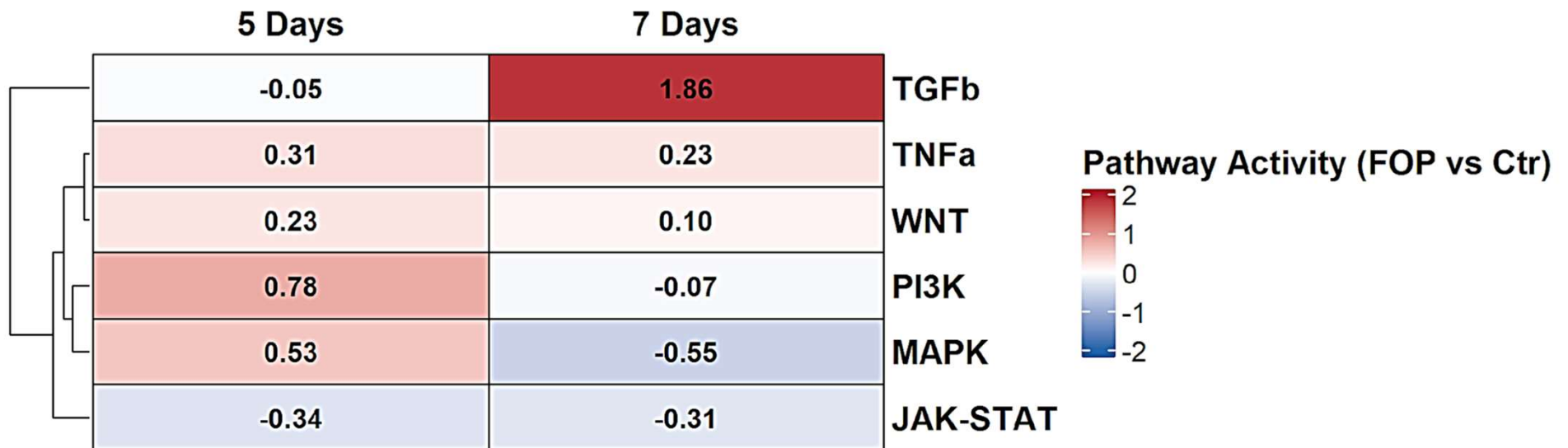
Spp1 interacts with CD44 and integrins



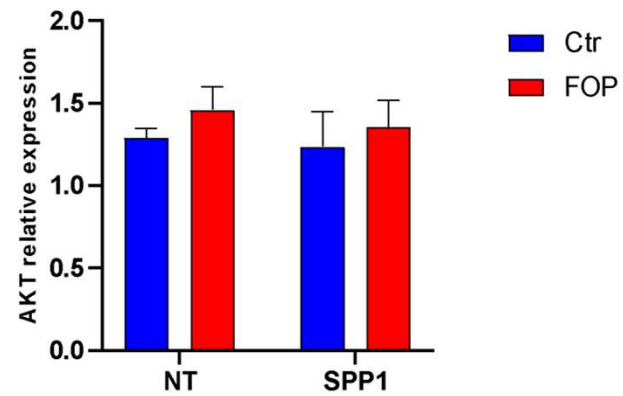
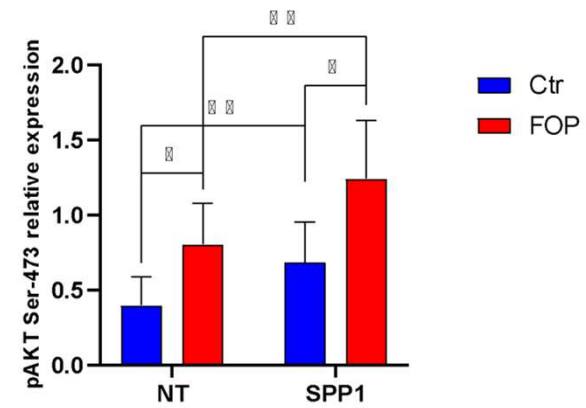
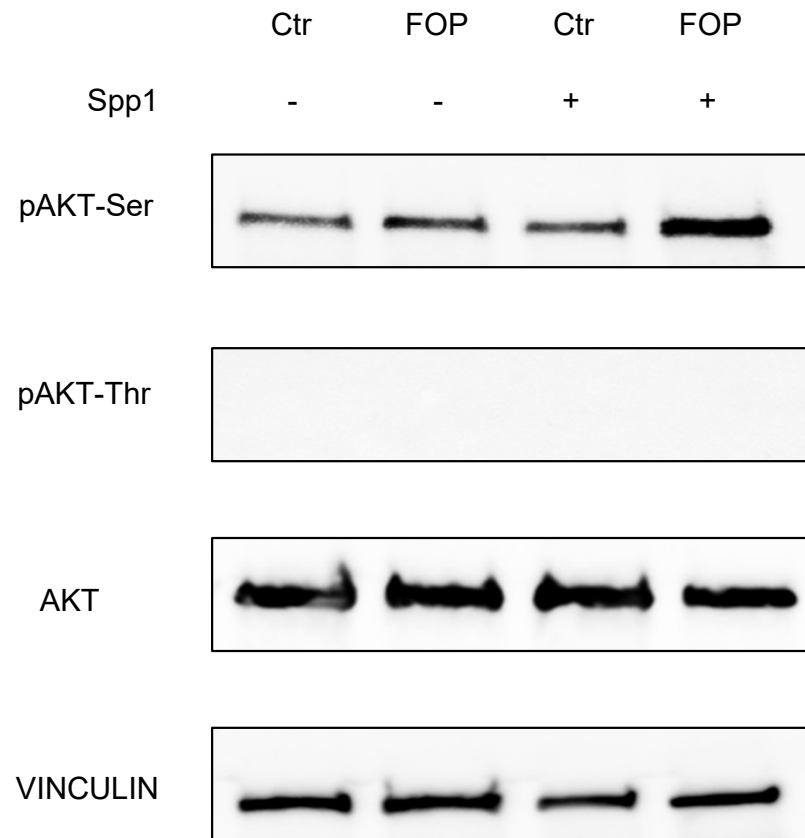
Jung et al. *Molecules and Cells*. 2025

Which are the pathways activated in FOP FAPs and MPs?

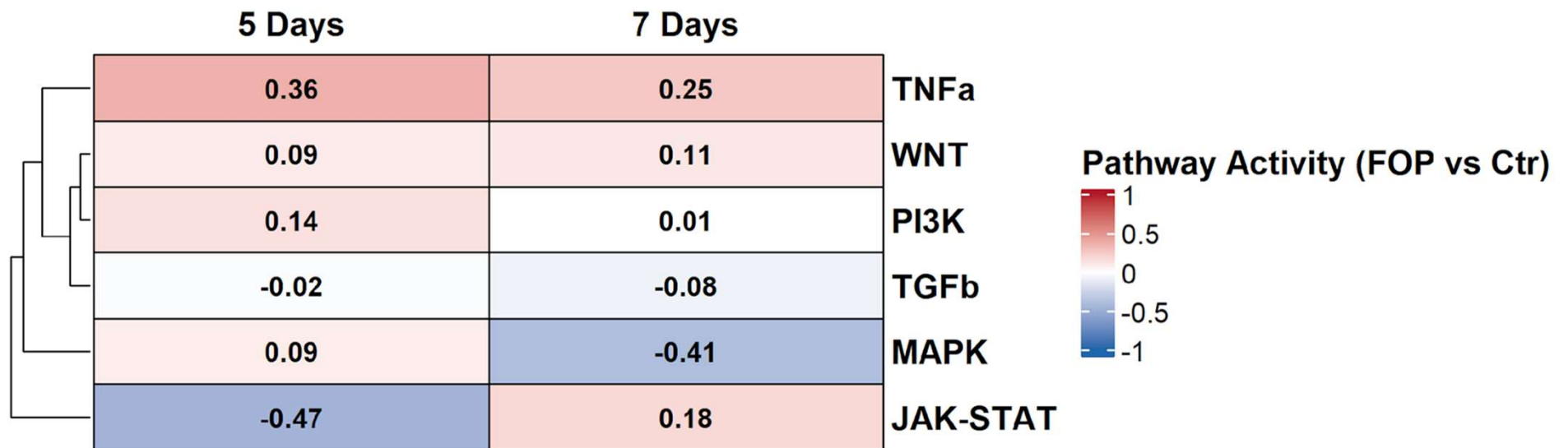
PROGENy analysis show altered pathways activated in FOP FAPs



Spp1 leads to increased of pAKT-Ser in FOP FAPs



PROGENy analysis show altered pathways activated in FOP MPs



Acknowledgements



**School of Medicine and Surgery
University of Milano Bicocca
HEVA RESEARCH GROUP**

Mauro Bergamaschi
Riccardo Gamberale
Michela Signo
Emanuele Azzoni
Raffaella Meneveri
Cristiana Baroni
Filipa Timoteo
Cristina D'Orlando



**Division of Immunology, S.
Raffaele Sc. Institute**
Angelo Manfredi
Patrizia Rovere-Querini

**Imaging Facility,
S. Raffaele Sc. Institute**
Antonello Spinelli

**DiNOGMI, University of
Genoa**
Istituto Gaslini
Roberto Ravazzolo
Renata Bocciardi
Serena Cappato

Université Claude Bernard Lyon 1, Lyon, France
Bénédicte Chazaud

Università Federico II, Napoli
Paolo Netti
Francesco Urciolo

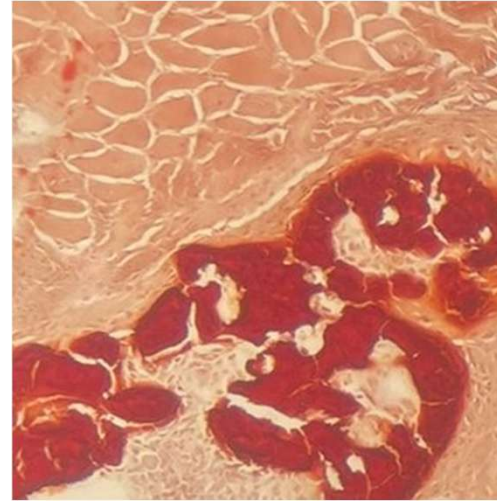
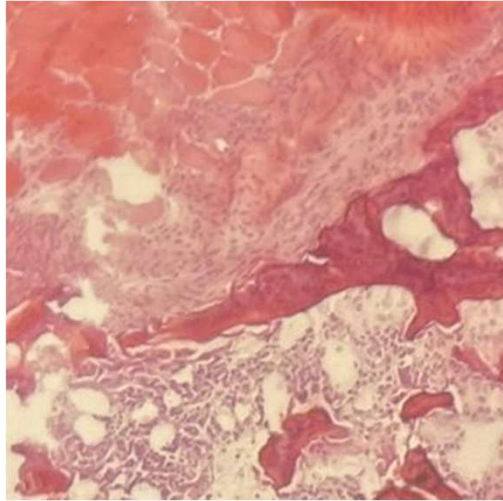
**Center for Omics Sciences
S. Raffaele Sc. Institute**
Dejan Lazarevic
Anna Sofia Tascini



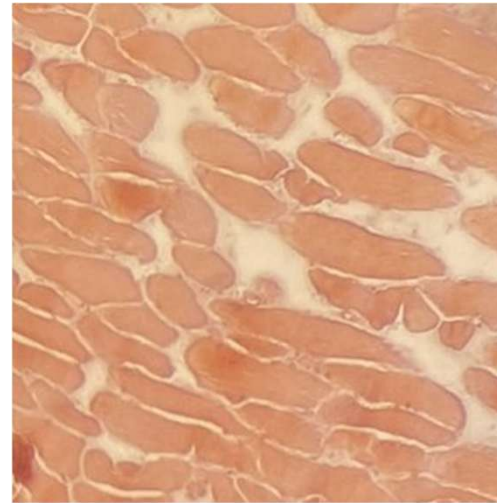
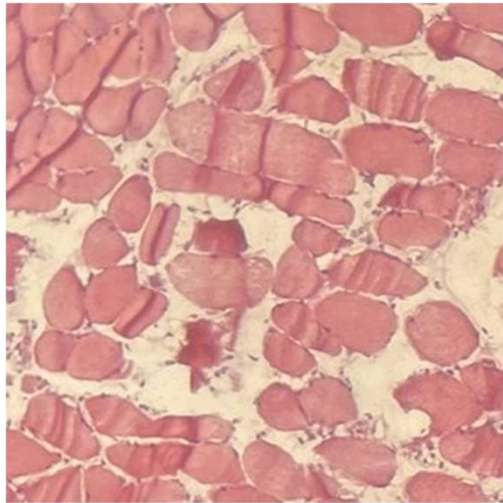
Hematoxylin Eosin

Alizarin Red

Vehicle



OPNi



SPP1 leads to ActivinA and MMP9 overexpression in FOP FAPs

